EVALUATION OF ALTERATIONS IN AUTONOMIC NERVOUS SYSTEM (HRV ANALYSIS) AND CENTRAL NERVOUS SYSTEM (VEP) IN TYPE 1 DIABETES MELLITUS PATIENTS

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CERTIFICATE

This is to certify that this dissertation entitled **"EVALUATION OF ALTERATIONS IN AUTONOMIC NERVOUS SYSTEM (HRV ANALYSIS) AND CENTRAL NERVOUS SYSTEM (VEP) IN TYPE 1 DIABETES MELLITUS PATIENTS"** by the candidate **Dr. M. JANET SUGANTHA** for M.D (Physiology) Branch - V is a bonafide record of the research work done by her, under the guidance of **Dr. K. BALASUBRAMANIAN M.D.** Professor, Head of the Department of Physiology, Stanley Medical College, during the period of study (2008 - 2011), in the Department of Physiology, Stanley Medical College, Chennai – 600 001.

I also certify that this dissertation is the result of the independent work on the part of the candidate

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INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-3

Title of the Work	:	Visual evoked potentials & Automatic function tests in type I diabetic melitus
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The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 03.08.2010 at the Modernised Seminar Hall, Stanley Medical College, Chennai-1 at 2PM

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LIST OF ABBREVIATIONS

ANS	Autonomic Nervous System
AR	Auto regressive
AV	Atrio Ventricular
bpm	beats per minute
CAN	Cardiac Autonomic Neuropathy
CNS	Central Nervous System
DAN	Diabetic Autonomic Neuropathy
db	deep breathing
DM	Diabetes Mellitus
ECG	Electrocardiogram
EP	Evoked Potentials
FFT	Fast Fourier Transform
HF	High Frequency
HR	Heart Rate

HRV	Heart Rate Variability
IDDM	Insulin Dependent Diabetes Mellitus
LF	Low Frequency
MI	Myocardial Infarction
ms^2	millisecond square
NN50	Normal to Normal RR interval deviation more than 50ms
n.u.	normalized units
PNS	Parasympathetic Nervous System
PSD	Power Spectral Density
RHR	Resting Heart Rate
RMSSD	Root Mean of the Sum of Squares of Difference between adjacent NN intervals
SA	Sino Atrial node
SBP	Systolic Blood Pressure

SD	Standard Deviation
SDNN	Standard Deviation of average Normal to Normal RR intervals
SMC	Stanley Medical College
SNS	Sympathetic Nervous System
VDU	Visual Display Unit
VEP	Visual Evoked Potentials
VLF	Very Low Frequency

INTRODUCTION

Type 1 Diabetes mellitus previously referred to as ‘juvenile-onset’ or ‘insulin dependent’ diabetes, most commonly develops in childhood and accounts for 5 to 15% of all cases of diabetes.

AETIOPATHOGENESIS

Type 1 Diabetes is caused by an autoimmune, predominantly T-cell mediated process that selectively destroys the pancreatic β cells.

Genetic factors explain 30 to 40% of total susceptibility: at least 10 loci are involved, with the HLA class II locus IDDM having by far the greatest effect.

Environmental factors that have been implicated include viral infection (particularly Coxsackie B), bovine serum albumin from cow’s milk (by immunological cross-reactivity) and other toxins.

Although type 1 DM most commonly develops before the age of 30, an autoimmune beta cell destructive process can develop at any age. Several years of progressive autoimmune damage usually precede the clinical onset of diabetes.

CLINICAL FEATURES

Classical presentation of untreated or poorly controlled type 1 diabetes is with onset over days or a few weeks of polyuria (caused by osmotic diuresis due to hyperglycaemia), thirst, weight loss and general tiredness/ malaise. Other features can include blurred vision (due to hyperglycaemia- related refractive changes in the lens), infection (particularly genital candidiasis) and diabetic ketoacidosis.

DIAGNOSIS

The National Diabetes Data Group and World Health Organization[1] have issued diagnostic criteria for Diabetes mellitus-

- Symptoms of diabetes plus random blood glucose concentration ≥11.1mmol/L, (200 mg/dl) or
- Fasting plasma glucose ≥7.0mmol/L (126mg/dl) or
- Two-hour plasma glucose ≥ 11.1 mmol/L (200mg/dl) during an oral glucose tolerance test.

ACUTE COMPLICATIONS

Diabetic Ketoacidosis

CHRONIC COMPLICATIONS

The chronic complications of DM affect many organ systems and are responsible for the majority of the morbidity and mortality associated with the disease.

MICROVASCULAR COMPLICATIONS:

Retinopathy

Neuropathy

- Sensory and motor
- Autonomic

Nephropathy

MACROVASCULAR COMPLICATIONS:

Coronary artery disease

Peripheral arterial disease

Cerebrovascular disease

OTHERS:

Gastroparesis

Infections

Skin changes

MECHANISMS OF COMPLICATIONS:

Four prominent theories have been proposed to explain how hyperglycemia might lead to the chronic complications of DM.

One theory is that increased intracellular glucose leads to the formation of advanced glycosylation end products (AGEs) via the nonenzymatic glycosylation of intra- and extracellular proteins. The serum levels of AGEs correlates with the level of glycemia, and these products accumulate as glomerular filtration rate declines.

A second theory is based on the observation that hyperglycemia increases glucose metabolism via the sorbitol pathway. Increased sorbitol concentration alters redox potential, increases cellular osmolality, generates reactive oxygen species, and likely leads to other types of cellular dysfunction.

A third hypothesis proposes that hyperglycemia increases the formation of diacylglycerol leading to the activation of protein kinase C (PKC). PKC alters the transcription of genes for fibronectin, type IV collagen, contractile proteins and extracellular matrix proteins in endothelial cells and neurons.

A fourth theory proposes that hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate. The hexosamine pathway may alter function by glycosylation of proteins such as

endothelial nitric oxide synthase or by changes in gene expression of transforming growth factor β or plasminogen activator inhibitor-1.

Growth factors appear to play an important role in DM-related complications, and their production is increased by most of these proposed pathways.

A possible unifying mechanism is that hyperglycemia leads to increased production of reactive oxygen species or superoxide in the mitochondria; these compounds may activate all four of the pathways described above. Although hyperglycemia serves as the initial trigger for complications of diabetes, it is still unknown whether the same pathophysiologic processes are operative in all complications or whether some pathways predominate in certain organs.

PROGNOSIS

The main threat to survival with type 1 diabetes is chronic tissue damage, particularly renal failure from nephropathy, vascular disease notably myocardial infarction and stroke.

Throughout adult life, the overall risk of dying within 10 years is about fourfold higher for patients with type 1 diabetes than for their non diabetic peers.

Neuropathy is a common chronic complication of Diabetes mellitus.

It affects the peripheral nerves, autonomic nerves as well as the Central nervous system. In this study, I have attempted to evaluate the Cardiovascular Autonomic functions and Central nervous system alterations.

TYPE 1 DIABETES AND AUTONOMIC NEUROPATHY

The unconscious neural control of the body's physiology is effected through the Autonomic Nervous System. This innervates the cardiovascular and respiratory systems, smooth muscle of the gastrointestinal tract and glands throughout the body. The Autonomic system is controlled centrally by diffuse modulatory systems in the brainstem, limbic system and frontal lobes which are concerned with arousal and background behavioural responses to threat. The output of the Autonomic system is divided functionally and pharmacologically into two divisions: The Parasympathetic and Sympathetic systems.

SYMPTOMS OF AUTONOMIC NEUROPATHY:

A common symptom is an excessive decrease in blood pressure when the person stands (orthostatic hypotension). As a result, the person feels light-headed or as if about to faint. Men may have difficulty initiating and maintaining an erection (erectile dysfunction). Some people involuntarily pass urine (urinary incontinence), often because the bladder is overactive. Other people have difficulty emptying the bladder (urine retention) because the bladder is underactive. After eating, some people feel prematurely full or even

vomit because the stomach empties slowly (gastroparesis). Severe constipation may occur.

Individuals with long standing type 1 DM may develop signs of autonomic dysfunction. DM-related autonomic neuropathy can involve multiple systems including the cardiovascular, gastrointestinal, genitourinary, sudomotor and metabolic systems.

Autonomic neuropathies affecting the cardiovascular system cause a resting tachycardia and orthostatic hypotension. Reports of sudden death have also been attributed to autonomic neuropathy.

AUTONOMIC REGULATION OF CARDIO VASCULAR FUNCTION

Autonomic nervous system through the autonomic reflex that regularly responds to internal and external stimuli modulate the activity of the body's organs. ANS like any other somatic nervous system, is well organized on the basis of reflex arc. ANS depends on the functional integrity of multiple autonomic reflex arc. In simple terms the ANS consist of afferent path ways, central processing system and efferent pathways; ANS out flow is divided into two components. The throacolumbar sympathetic out flow and craniosacral parasympathetic out flow, whose effects are mostly antagonistic and whole balance activates to produce homeostasis in any system. The peripheral out flow of the ANS are brought out by preganglionic and postganglionic nerves.

AUTONOMIC INNERVATIONS OF CARDIO VASCULAR SYSTEM

The heart and vascular system are innervated by both parasympathetic and sympathetic nerves. The predominant supply of vagus is to the pace maker and conducting system and the sympathetic supply is more for cardiac muscle and vascular system. So the changes in the heart rate are predominantly modulated by the vagus and the contractility of cardiac muscle is brought about by sympathetic pathway. Although some local factors, such as temperature, hormone changes and stretch of tissues can change the heart rate, the ANS is the principle way by which the heart rate can be controlled effectively. We understand that the average resting HR is 70 beats/min in normal adults at rest and is very much greater in fetus and in children. During sleep the HR decreases around 10-20 beats/min and during emotional states and muscular exercise (any stress) the HR may go upto twice that of the resting HR or more. The HR increase is due to the decrease in PNS and with increase in SNS. Though the two nerves act on SA node, the vagus is predominant in healthy and resting individuals. When healthy individual is given atropine, a muscarinic receptor antagonist that blocks parasympathetic (PNS) effects, the heart rate increases sufficiently. If a healthy person is given propanolol, a β adrenergic receptor antagonist that blocks SNS, the heart rate decreases only slightly. It is interesting to note that when both divisions of ANS is blocked, the heart rate in young adults averages about 100 beats/min (Ganong WF)[2]. The rate that

prevails after complete ANS blockade is called the intrinsic heart rate. This is the most important concept that we have to understand. During the physiological stress however there is decreased parasympathetic activity and relative or absolute increase in sympathetic activity that causes changes in the cardio-vascular function.

AUTORHYTHMICITY

The heart has its own inherent activity which is seen in spontaneously firing pacemaker cells in the wall of the right atrium. These cells are specialized cardiac muscle cells with scanty contractile fibers with large number of gap junctions. These cells have their membrane potential that after each impulse comes back to firing level what is called as **prepotential** (or) **pace maker potential**. At the peak of each impulse, potassium IK efflux begins and brings about repolarization. IK then declines, and as efflux decreases, the membrane begins to depolarize, forming the first part of prepotential, Ca2+ channels then open. The calcium current (ICa) due to opening of 'T' (Transient channels) complete the prepotential followed by entry through 'L' (Long lasting) channels to produce the impulse.

PARASYMPATHETIC PATHWAY

The cardiac parasympathetic fibers originate in the medulla oblongata cells that lie in the dorsal motor nucleus of the vagus (or) the nucleus ambiguus.

In humans the vagal fibers pass through the neck close to the common carotid arteries and then through the mediastinum to synapse with the post ganglionic cells. The vagal post ganglionic pathways go across the right atrial epicardium to the SA node. These cells are located on the epicardial surface within the wall of the heart. Most of the cardiac ganglion cells are located near the SA node and atrioventricular (AV) conduction tissue. The right vagus nerve affects predominantly SA node. Stimulation of this nerve slow SA nodal firing and can even stop it for several seconds. The left vagus nerve mainly inhibits AV conduction tissue to produce various degrees of AV blocks. But there is also overlapping of left and right vagal fibers. Because of this overlapping, left vagal stimulation also depress the SA node, and right vagal stimulation impede the AV conduction. Among right and left vagus, the right vagus predominately dominates the pacemaker cells of the heart in determining the heart rate changes.

SYMPATHETIC PATHWAY

The cardiac sympathetic fibers originate in the intermediolateral horn cells of the upper six thoracic segments and lower two cervical segments of the spinal cord. The fibers emerge from the spinal column through white communicating branches and enter para vertebral chains of ganglia. The preganglionic and post ganglionic nerves synapse mainly in the stellate (or) middle cervical ganglia. In the mediastinum, the post ganglionic sympathetic

fibers and preganglionic parasympathetic fibers join to form a complex plexus of the mixed efferent nerves to the heart. The post ganglionic cardiac sympathetic fibers in this plexus approach the base of the heart along the adventitial surface of the great vessels. On entering the base of the heart, the fibers are distributed to the various chambers as an extensive epicardial plexus. They then penetrate the myocardium along the coronary vessels. The left side sympathetic fibers are distributed to left side of heart having predominant role on the myocardial contractility. The right side sympathetic distribution carries the changes in the heart rate. There is also extensive crossing over of these fibers.

In the present study I have sincerely attempted to find out the Cardio-vascular Autonomic function in Type 1 Diabetic patients. The recent availability of sensitive, specific and reproducible noninvasive tests of autonomic function has enhanced our understanding of the prevalence, pathophysiology and clinical manifestations of this disorder. Heart rate variability is a simple non invasive test to assess the autonomic balance.

HEART RATE VARIABILITY (HRV)

INTRODUCTION

HRV refers to the beat to beat alteration in heart rate, i.e., the oscillation in the interval between consecutive heart beats as well as the oscillations

between consecutive instantaneous heart rate. "**Heart rate variability (HRV)**" has become the conventionally accepted term to describe variations of both instantaneous heart rate and RR intervals. To describe oscillation in consecutive cardiac cycles, other terms have been used in the literature, for example**, cycle length variability, heart period variability, RR variability, and RR interval tachogram.** These terms have not gained as wide acceptance as HRV; hence, I have used the term HRV in this dissertation.

We know that the cardiac automaticity is intrinsic to pacemaker tissue; heart rate and rhythm are largely under the control of the autonomic nervous system. Studies in the last 30 years have shown a significant relationship between ANS and Cardio-vascular morbidity, including sudden cardiac death[3]. Heart rate variability is a non-invasive estimate of the function of sympathetic and parasympathetic nervous system. It is a window for analyzing ANS. At present many commercial devices provide automated measurement of HRV. Physiologists and clinical cardiology research scholars have made use of these instruments, but the precise measurement and meaning of HRV analysis are more complex.

European Society of Cardiology and North American Society of Pacing and Electrophysiology have constituted a Task force to develop appropriate standards. In this study, I have followed the recommendations and guide lines of the Task force 1996 (Circulation 1996)[4]

MEASUREMENT OF HRV

The understanding of Autonomic function on the basis of RR interval fluctuations in the heart rate is by two methods.

- Time domain methods.
- Frequency domain methods

TIME DOMAIN METHOD

The variations in heart rate can be evaluated by a number of methods. Perhaps the simplest to perform are the time domain measures. In these methods, either the heart rate at any point in time or the intervals between successive normal QRS complexes are determined. In a continuous ECG record, each QRS complex is detected, and the so-called normal-to normal (NN) intervals (that is, all intervals between adjacent QRS complexes resulting from sinus node depolarizations) or the instantaneous heart rate is determined. Simple time domain variables that can be calculated include the mean NN interval, the mean heart rate, the difference between the longest and shortest NN interval, the difference between night and day heart rate, and so forth. Other time domain measurements that can be used are variations in instantaneous heart rate secondary to respiration, tilt, Valsalva maneuver, or phenylephrine infusion. These differences can be described as either differences in heart rate or cycle length.

TIME DOMAIN MEASURES OF HRV

Variable	Units	Description
SDNN	ms	Standard deviation of all NN intervals
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50ms in the entire recording
pNN50	%	NN50 count divided by the total number of all NN intervals

FREQUENCY DOMAIN METHOD

Various spectral methods[5] for the analysis of the tachogram have been applied since the late 1960s. Power spectral density (PSD) analysis provides the basic information of how power (variance) distributes as a function of frequency[6]. Independent of the method used, only an estimate of the true PSD of the signal can be obtained by proper mathematical algorithms. They are

1) Non-parametric method

2) Parametric method

The advantages of the nonparametric methods are

(1) The simplicity of the algorithm used (fast Fourier transform [FFT] in most of the cases) and

(2) The high processing speed.

The advantages of parametric methods are

(1) Smoother spectral components that can be distinguished independent of preselected frequency bands

(2) Easy post processing of the spectrum with an automatic calculation of low- and high-frequency power components with an easy identification of the central frequency of each component and

(3) An accurate estimation of PSD even on a small number of samples on which the signal is supposed to maintain stationarity.

The basic disadvantage of parametric methods is the need of verification of the suitability of the chosen model and of its complexity (that is the order of the model). In my study, I have used the non parametric method for calculating the Power Spectral Density.

Three main spectral components[7] are distinguished in a spectrum calculated from short term ECG recordings – VLF (Very Low frequency), LF(Low frequency) and HF (High Frequency).

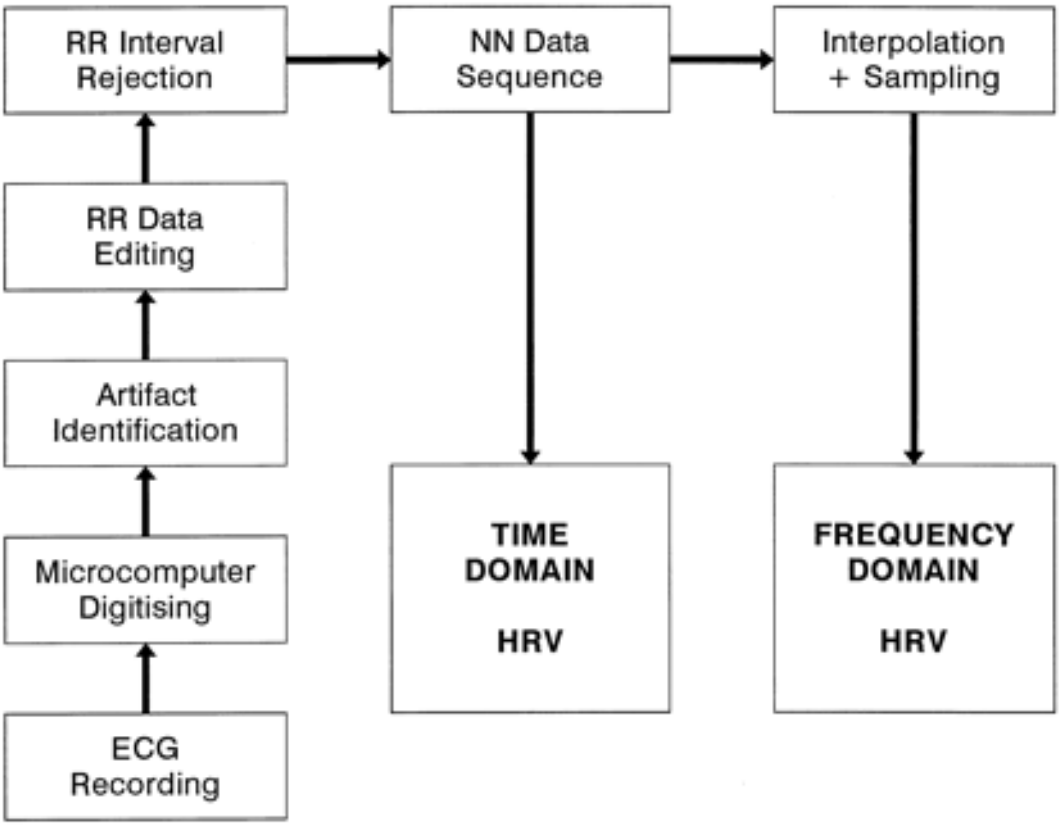
FREQUENCY – DOMAIN MEASURES

Variable	Units	Frequency	Significance
VLF	ms^2	4-40 mHz	Temperature changes Renin Angiotensin System
LF	ms^2	40-150 mHz	Tracks baroreflex mediated RR oscillation and centrally generated RR oscillation. Sympathetic > Parasympathetic
HF	ms^2	150-400 mHz	A reliable index of Vagal Modulation of RR interval. Only Parasympathetic.
LF/HF			Sympatho vagal balance

LF and HF may also be measured in normalized units[8,9] which represent the relative value of each power component in proportion to the total power minus the VLF component. The representation of LF and HF in normalized units emphasizes the controlled and balanced behaviour of the two branches of the ANS. Moreover, the normalization tends to minimize the effect of the changes in Total Power on the values of LF and HF components.

In this study, I have used only short term HRV that is done for 5 mins.

GENESIS OF HRV



RECORDING REQUIREMENTS

ECG SIGNAL

The fiducial point recognized on the ECG tracing that identifies a QRS complex may be based on the maximum or baricentrum of the complex, on the determination of the maximum of an interpolating curve, or found by matching

with a template or other event markers. To localize the fiducial point, voluntary standards for diagnostic ECG equipment are satisfactory in terms of signal-to-noise ratio, common mode rejection, bandwidth, and so forth. An upper-band frequency cutoff substantially lower than that established for diagnostic equipment (≈200 Hz) may create a jitter in the recognition of the QRS complex fiducial point, introducing an error of measured RR intervals. Similarly, limited sampling rate induces an error in the HRV spectrum that increases with frequency, thus affecting more high-frequency components. An interpolation of the undersampled ECG signal may decrease this error. With proper interpolation, even a 100-Hz sampling rate can be sufficient

TYPE 1 DIABETES AND CENTRAL NERVOUS SYSTEM ALTERATIONS

Impairment of the Central Nervous system is a frequent complication of diabetes, but its clinical importance is still underestimated. The exact pathophysiology of the central nervous dysfunction is not clear, but it seems to be multifactorial, involving vascular and metabolic factors, similar to the pathogenesis of diabetic peripheral neuropathy. Earlier studies revealed new data on the central manifestations of diabetes but did not permit a comprehensive comparative analysis of the peripheral and central neuronal dysfunction

Evaluation of the Visual Evoked potentials furnishes a diagnostic tool for the assessment of functional anomalies of the cerebral function, even at an early stage of the pathogenetic process. The aim of my study is to assess the CNS alterations using Visual Evoked Potentials (VEP) and to demonstrate the possible associations between the latencies of VEP and the severity of the cardiovascular autonomic dysfunction in type 1 diabetes.

INTRODUCTION TO VISUAL EVOKED POTENTIALS

Visual evoked potentials (VEPs) are electrical potential differences recorded from the scalp in response to visual stimuli. Normal cortical responses are obtained if the entire visual system is intact and disturbances anywhere in the visual system can produce abnormal VEPs. Therefore the localizing value of VEP is limited.

METHODS AND INSTRUMENTATION

BASIC INSTRUMENTATION FOR DATA ACQUISITION

1. AMPLIFIERS

Equipment for recording EPs must be suitable for amplifying electrical signals with amplitudes down to 0.1 micro volts and band width from below 1 Hz to 10 KHz.

The first stage of amplification uses pre-amplifiers which can be placed close to the patient so that the leads to the electrodes can be kept short and the cable between the pre and main amplifiers carries signals from a low impedance source– both features help to reduce pick–up mains and other interferences. The impedance of the skin electrodes is less than 10kΩ and the input impedance of the amplifiers 10mΩ or more.

The amplifiers have a wide range of stepped gain control.

2. FILTERS

Both low and high pass filters have a number of set positions so that the upper frequency limit can be reduced from 10 or 20 KHz to 30 Hz and the lower frequency limit raised from 0.01 Hz to 300 Hz.

3. ELECTRODES

Electrodes are attached to the skin by means of a special paste, which serves as an adhesive, but also establishes a good contact with the skin. Electrodes made from dissimilar metals should be avoided. A system is available to measure the impedance of each electrode.

4. STIMULATORS

All EP investigations require sensory stimulation. Stimulators are an integral part of the equipment. The stimuli are synchronized to the sampling epoch and the trigger point advanced or delayed from the start of the epoch to facilitate viewing the data on the screen.

5. DISPLAY

The EPs are displayed on an oscilloscope after averaging. The data are manipulated by digital techniques before being displayed in colour on a VDU. Further adjustment can be done in the display mode before the data are stored.

Several traces can be displayed on the VDU at the same time, so that comparisons can be made. Latencies and amplitudes are obtained from the displayed data either automatically or by cursor measurements.

6. VISUAL STIMULATION

FLASH: The most used type of visual stimulation is the stroboscope flash. The stroboscope produces an intense, brief pulse of light and is used in clinical neurophysiology to evoke the VEP. It can be used as a single flash or repetitively.

PATTERNS & PICTURES

Stationary pictures or patterns can be projected onto a screen. High quality pictures can be displayed on a monochrome or colour VDU under software control. Moving or changing patterns such as checker board reversal can be generated on a VDU and switched electromechanically at the required rate (2/sec). The latency of the pattern reversal EP is dependent on the luminance of the pattern.

RECORDING PROCEDURE

VEPs can be evoked by brief changes either in the luminance or in the pattern within the field of vision; both types of changes can also be combined in a single stimulus.

ANALYSIS TIME & SAMPLING RATES

Scalp VEPs are obtainable in all normal adults peak with latencies in 70-150 msec. range. Consequently an analysis time of 300 msecs is suitable. In children a slightly longer analysis time, upto 500 msecs is used.

FILTERS

Filters are set between 1 to 100 Hz. The filters must be kept constant in the laboratory for the recording of the control subjects and the patients.

SENSITIVITY & NUMBER OF SWEEPS

A recording sensitivity of 200 micro volt/cm and averaging 100 sweeps are conditions for recording flash and full field pattern VEP.

ELECTRODE PLACEMENT AND MONTAGES

An active electrode placed at Oz, or 5cm above the inion on the midline, picks up VEPs with the maximum amplitude.

A reference electrode is positioned 12 cm above the bridge of the nose.

The ground electrode is placed in the midline in forehead.

SPECIAL CONTROLS FOR RUNNING VEP TEST

GAZE FIXATION

Recording VEPs requires active co-operation of the subject who is instructed to gaze at a dot at the center of the pattern. The most elaborate means of controlling fixation is to record eye movements and automatically reject all sweeps contaminated by eye movement potentials. Patients with visual defects involuntarily shift their gaze into the good visual field, a phenomenon related to the formation of a pseudo fovea. Thus they will have a small response to stimulation of the blind half field when the fixation dot is placed at the edge of the pattern.

MONO OCULAR STIMULATION

Monocular stimulation is obtained by covering the non stimulated eye with the patch. This patch must be made of opaque material and light should not leak around its edges.

NORMAL FINDINGS IN VEP

Pattern VEPs contain 3 main components, labelled N75, P100 & N145 which are recorded in the mid occipital region when the pattern is presented in the central part of the visual field, when it subtends an angle of 5 degrees at the eye.

Of the 3 components, the positive one peaking at the latency of 100 msec(P100) has the largest amplitude and can be obtained with a wide range of patterns although its amplitude and latency are affected by changing the stimulus parameters. Hence the clinical interpretation of pattern VEPs is based mainly on the measurement of P100 latency.

LATENCY AND AMPLITUDE MEASUREMENT

The latency of P100 potential is universally accepted as the most useful measure for interpreting the pattern VEPs. P100 latencies from 90-120msec have been reported in normal subjects with S.D not exceeding 8% of the mean.

The difficulties encountered in choosing the point at which the peak of P100 should be taken are:

1. Irregular shape of the component in abnormal wave forms.
2. The blind pattern of this peak in some subjects.
3. Identification of the peak when it is delayed.

Irregular shape of P100 results from the admixed noise & super imposition of 2 or 3 averaged traces recorded in the same conditions is the most reliable means of differentiating the peak from the background noise.

The bifid pattern of the P100 peak is encountered when stimulating with bright or highly contrasted checker board. This can be prevented by reducing

the contrast between bright and dark squares to 50% or 20%. This causes the latter of the 2 positivities to disappear.

AMPLITUDE MEASUREMENTS

The amplitude of the P100 potential measured from base line or from the peak of the N75 negativity, shows the greater inter-individual variability. Values between 2-20 microvolts fall into the normal range of the most laboratories and the study of interocular differences is crucial for interpreting the wave forms.

INTEROCULAR DIFFERENCES OF LATENCIES AND AMPLITUDES

In healthy individuals the latencies and amplitudes of the P100 potentials from each eye are almost identical. Interocular P100 latencies and amplitude differences over 10msec & 8microvolts respectively are reported as beyond the upper limits of normality as is an amplitude ratio greater than 2:3.

NONPATHOLOGICAL SOURCES OF VEP VARIATION

SUBJECT FACTORS:

AGE:-

The P100 latency is decreased in children and adult values are reached only after 5 years. There is absence of age related changes of the P100 latency in adults until the 5^{th} decade. After the 5th decade the mean value and the variance

of the P100 latency increases with the age in females between 50- 70 years whereas in the male it remains fairly stable. No major changes occur in the amplitude of P100 during adult life.

GENDER:-

P100 has shorter latency and greater amplitude in female than in males within the 20 to 50 years age range. Due to the increase of P100 latency in females over 50 years the sex difference were not obvious beyond this age.

VISUAL ACUITY: -

With a checkerboard containing large checks, P100 latency is insensitive to refractive errors, but with smaller checks represented foveally, it increases when the retinal image is defocused. Therefore visual acuity must be measured and refractive errors corrected before recording VEPs and patients should wear glasses during testing. Poor visual acuity causes prolonged and low amplitude pattern VEPs.

BODY TEMPERATURE AND PHYSICAL EXERCISE

Temperature does not affect the latency or amplitude of P100. Physical exercise immediately before VEP recording produces significant reduction of P100 amplitude.

ATTENTION AND ACCOMODATION

When the subject is engaged in a mental task not related to visual stimulus, there is no significant change in P100 latency.

TECHNICAL FACTORS:

LUMINANCE

There is a decrease in amplitude and increase in the latency of 18% and 15 msecs. respectively log unit diminution of the mean luminance of the screen.

STIMULATOR TYPE

The P100 latency is influenced by the time taken to reverse the pattern. An increase of 1 msec. in the reversal time causes a 0.6 msecs increase of P100 latency.

REVIEW OF LITERATURE

HRV

- Galen(1528) showed sympatho vagal trunk
- Etienne'Stephamis(1545) found out that Vagus and Sympathetic nerves were separate nerves
- Whytts (1752) proved the essence of reflex actions
- Weber & Weber 1845 showed that HR is reduced by vagal stimulation and heart rate is increased by sympathetic stimulation
- Langley (1898) introduced Autonomic Nervous System
- Alquist (1948) found the receptors and Sympathetic nervous system
- Hon and Lee (1965) [10] noted the fetal heart rate changes during fetal distress
- Ewing et al [11] devised a number of simple bed side tests of short term RR differences to detect autonomic neuropathy in diabetic patients.
- Wolf et al (1978) [12] proved that reduced HRV is a risk factor of sudden death in post MI patients
- Akselrod et al(1981) [13] introduced Power Spectral analysis of heart rate fluctuations to quantitatively evaluate beat-to-beat cardiovascular control.
- Knuttgen et al (1990)[14]showed Diabetic autonomic neuropathy (DAN) as a risk factor for surgical procedures. Cardiovascular autonomic function was examined preoperatively by a combination of tests (heart rate variations

during deep breathing, Valsalva ratio, 30:15 ratio, postural hypotension, sustained hand grip).

- Bernardi et al (1992) [15] showed that impaired circadian modulation of sympathovagal activity occurs in diabetes.
- Montano et al (1994) [16] noted that Spectral analysis of HRV, using nu or LF-to-HF ratio, appears to be capable of providing a noninvasive quantitative evaluation of graded changes in the state of the sympathovagal balance during graded orthostatic tilt
- Latson et al(1994) [17] showed that autonomic reflex dysfunction in patients with diabetes is associated with an increased incidence of hypotension after induction of anesthesia
- Dariusz Korczak et al(1997) [18] concluded that 1) in IDDM patients cardiac autonomic dysfunction occurs frequently and especially concerns impairment of the parasympathetic activity 2) cardiac autonomic dysfunction is more expressed in patients with diabetic neuropathy 3) heart rate spectral analysis is a valuable method for the cardiac autonomic function monitoring in patients with IDDM
- Karamitsos et al (1998) [19] showed that HRV indices are the earlier markers of DAN deterioration
- Martial M. Massin, MD et al(1999) [20] found evidence of early cardiac autonomic neuropathy in young patients with type 1 diabetes, even in

patients with good metabolic control. Time domain parameters normalized for the mean RR interval, especially those representing the vagal activity and the balance LF/HF, are the first indices of cardiac dysautonomia in young diabetic patients.

- Pagani M. (2000)[21] found that normalized units provide an estimate of the balance between sympathetic and vagal modulatory activity. In states of sympathetic predominance, such as during orthostatic positions, LF increases and HF decreases
- Kitamura et al(2000)[22] showed that patients with diabetic neuropathy are at risk of a greater intraoperative reduction in core temperature
- Singh JP et al (2000)[23] HRV is inversely associated with plasma glucose levels and is reduced in diabetics as well as in subjects with impaired fasting glucose levels.
- Hejjel et al (2001)[24] proved that the beat-to-beat fluctuation of the heart rate originates from the momentary summing of sympathetic and parasympathetic influences on the sinus node
- Massimo Chessa et al (2002)[25] HRV analysis can detect early subclinical alterations of the autonomic nervous system in asymptomatic patients with IDDM, which seem to consist mainly in a parasympathetic impairment. Autonomic dysfunction is associated both with the duration and an inadequate metabolic control.

- Aaron I.Vinik et al(2003)[26] showed that reduced cardiovascular autonomic function as measured by HRV is strongly associated with an increased risk of silent myocardial ischemia. Measurement of HRV at the time of diagnosis of type 2 diabetes and within 5 years after diagnosis of type 1 diabetes serves to establish a baseline, with which 1-year interval tests can be compared. Regular HRV testing provides early detection and thereby promotes timely diagnostic and therapeutic interventions. HRV testing may also facilitate differential diagnosis and the attribution of symptoms (e.g., erectile dysfunction, dyspepsia, and dizziness) to autonomic dysfunction.
- Colberg SR(2003)[27] found that individuals with diabetic autonomic neuropathy (DAN) exhibit an increased resting heart rate but depressed maximal heart rate.
- Migliaro et al (2003)[28] short-term studies are as powerful as Holter to differentiate the diabetic group (impaired HRV) from the control group.
- Sztajzel et al(2004)[29] proved the use of HRV as a marker reflecting the activity of the sympathetic and vagal components of the ANS on the sinus node, and as a clinical tool for screening and identifying patients particularly at risk for cardiac mortality
- Faulkner MS et al(2005) [30] found that incidence rates of both type 1 and type 2 diabetes mellitus (DM) are increasing in youth and may eventually contribute to premature heart disease in early adulthood

- Javorka M et al(2005)[31] found that abnormalities in cardiac parasympathetic regulation precede impairment of blood vessels sympathetic control in young diabetics
- Chemla D et al[32] The spectral components of short-term HRV calculated by using the FFT and AR methods were not interchangeable and FFT analysis must be preferred in diabetic patients.
- Khandoker et al(2008)[33] Cardiac autonomic neuropathy (CAN) in diabetes has been called a 'silent killer'
- Jakob R. Larsen, MD et al [34] Eighteen Years of Fair Glycemic Control preserves Cardiac Autonomic Function in Type 1 Diabetes.

VEP

- Cirillo D, et al (1984)[35] studied visual evoked potentials in 30 insulin-dependent diabetic children and adolescents. Thirty percent of the subjects had evidence of significant abnormalities.
- Anastasi M, et al, (1985)[36] studied the latency of pattern-reversal VEPs in type I insulin-dependent diabetics without retinal and extraocular involvement. The latencies of VEPs were progressively delayed in relation to the duration of the disease, becoming more and more evident and stabilizing after about 6 years from the onset of diabetes. The VEP

alterations probably indicate alteration of membrane balance or demyelinization.

- Ponte F, et al (1986)[37] studied VEPs elicited by pattern stimulation in a group of 62 type I insulin-dependent diabetics. The patients did not have retinopathy. An attempt was made to correlate the duration of the diabetes, insulin requirement, blood glucose and glycosylated hemoglobin with the VEPs. A positive correlation was found between the VEPs latency and the duration of the diabetes. No correlation was found with the other parameters considered.
- Khardori R, et al (1986)[38] recorded Brainstem auditory evoked potentials and pattern shift visual evoked potentials in 34 Type 1 (insulin-dependent) diabetic patients with long-standing disease and in 43 control subjects. Thirty-two percent of diabetic patients had abnormal brainstem auditory evoked potentials and 15% had abnormal visual evoked potentials. The findings of this study are consistent with a central diabetic neuropathy involving the brainstem in long-standing diabetic patients.
- Ponte F, et al (1986)[39] demonstrated abnormal latency delay of pattern-reversal visual evoked cortical potentials in insulin-dependent diabetics without retinopathy as a sign of subclinical damage.
- Comi G, et al (1987)[40] Peripheral neuropathy is a well-known complication of diabetes, but few data are available on central lesions. Visual evoked potentials (VEPs) seem a reliable and feasible technique for detecting a

conduction delay in the central nervous system. VEPs measurement seems a simple and reliable technique for detecting early alterations in CNS function in diabetics. They suggested that central and peripheral nervous alterations progress simultaneously.

- Lovasik JV, et al(1988)[41] examined the neural function of the retina by electroretinograms and the macular-cortical pathways by visual evoked potentials (VEP's) in 30 insulin-controlled juvenile diabetics and an age-and sex-matched group of nondiabetics.
- Palacz O,et al (1989)[42] Evoked visual potentials enable in a majority of cases to evidence the central and subclinical disturbances of the optic nerve.
- Mariani E, et al(1990)[43] Evaluated central optic pathways' involvement in diabetics, visual evoked potentials (VEP), in particular the latency of positive peak (LP100), were studied in 35 patients without retinopathy (4 insulin-dependent, 31 non-insulin-dependent) and 35 normal controls using reversal pattern stimulation. LP100 was significantly delayed in diabetics at both binocular and monocular stimulation.
- Aguggia M, et al (1993)[44] did a correlated study of visual evoked potentials-polyneuropathy in diabetic patients without retinopathy. Among 35 diabetic patients not suffering from retinopathy, that tested early deteriorations in visual pathways by means of pattern reversal VEPs and they considered similarities between these alterations, clinical metabolic parameters of the disease and clinical and paraclinical aspects of polyneuropathy

- Akinci A, et al (1994)[45] studied Brainstem auditory evoked potential, visual evoked potential and nerve conduction velocity and their relation with HbA1c and beta 2 microglobulin in children with insulin dependent diabetes mellitus.
- Ziegler O, et al (1994)[46] demonstrated improved visual evoked potential latencies in poorly controlled diabetic patients after short-term strict metabolic control.
- Uccioli L, et al (1995)[47] Electrophysiological assessment of visual function in newly-diagnosed IDDM patients. Visual evoked potentials (VEP) recorded under basal conditions showed that P100 latency was significantly increased in the diabetic patients compared to control subjects ($p < 0.01$), while N75-P100 amplitude was similar in both groups. The impaired basal VEPs suggest an early involvement of the nervous conduction in the optic nerve.
- Seidl R, et al(1996)[48] recorded brainstem auditory evoked potentials and visually evoked potentials in young patients with IDDM and concluded that EPs noninvasively detect subclinical central nervous system involvement in children and adolescents with IDDM
- Fierro B,et al (1996)[49] evaluated central nervous system involvement in diabetes, by investigating somatosensory (SEPs) and visual (VEPs) evoked potentials.

- Parisi V, et al (1997)[50] stated that electrophysiological impairment starts in the nervous conduction of the visual pathways with an early involvement, goes on in the innermost retinal layers and in the macula and ends in the middle and outer retinal layers.
- Parisi V,et al (1998)[51] assessed whether the VEP abnormalities are due to impaired function of the retinal layers and/or a delayed conduction in the postretinal visual pathways.
- Verrotti A, et al (2000)[52] suggested that early functional abnormalities of the optic nerve can be detected at onset of diabetes and that glycaemic control reverses these abnormalities.
- Das T, et al (2001)[53] concluded that subclinical CNS dysfunction is common in diabetes mellitus and this can be reliably detected by measuring the CNS latencies, specially VEP.
- Lopes de Faria JM, et al (2001)[54] noted a significant and nonselective neuronal visual loss involving the visual pathway that precedes the ophthalmoscopically detectable retinopathy in patients with type 1 DM.
- Varkonyi TT, et al (2002)[55] demonstrated the possible associations between the latencies of visual evoked potentials and the severity of the cardiovascular autonomic and peripheral sensory dysfunctions in type 1 diabetes. The prolongation of the P100 latencies of the visually evoked potentials for both eyes was associated with parasympathetic autonomic neuropathy and the hypesthetic form of lower limb sensory neuropathy.

- Szabela DA, et al (2005)[56] assessed the frequency of abnormal visual evoked potentials in type 1 diabetic patients and the correlation between patients' age, duration of the disease and metabolic control
- Elia YT, et al (2005)[57] examined the association between metabolic control (HbA(1c)) and the chromatic mechanisms of children with type 1 diabetes, by using the color visual evoked potential (VEP).
- Uzun N,et al (2006)[58] evaluated auditory, visual and sensorial abnormalities in type I diabetic patients, who also have normal nerve conduction studies, with somatosensory, brainstem auditory and visual EP studies (SEP, VEP, BAEP) and concluded that EP changes can be detected in asymptomatic patients that would be a predictor of future symptoms.
- Karlica D,et al (2010)[59] found that progressive increases in VEP latency values are a direct sign of retinal ganglion cell damage, which takes place even before the first ophthalmoscopically detectable signs of diabetic retinopathy arise.

AIM OF THE STUDY

To evaluate the Autonomic nervous system and Central nervous system alterations in Type 1 Diabetic patients.

OBJECTIVES

- To study the Cardiovascular Autonomic function in Type 1 Diabetics by doing Heart Rate Variability(HRV) analysis
- To study the alterations in Central nervous system function (Visual pathway) by measuring Visual Evoked Potentials(VEP)
- To compare the HRV and VEP parameters with that of healthy controls
- To assess if there is a correlation between the VEP and HRV parameters

MATERIALS AND METHODS

The study was conducted in the Neurophysiology Lab of the Department of Physiology, Stanley Medical College, Chennai.

CASES:

INCLUSION CRITERIA

- Type 1 Diabetes mellitus patients (based on WHO criteria) attending Diabetology out-patient department
- Age: 10 to 40 years, both gender
- Type 1 diabetic patients on Insulin treatment and with fairly good glycemic control
- Type 1 DM patients with duration of disease > 2 years
- Patients with normal visual acuity

EXCLUSION CRITERIA

- Smokers
- Alcoholics
- Hypertension, Coronary artery disease, Renal disorders, Thyroid disorders
- Patients with corneal opacity, squint, colour blindness
- Patients with history of autonomic dysfunction

CONTROLS

30 age and gender matched healthy subjects attending the Master health check up programme, SMC.

Study Design : Cross-sectional study.

The study protocol was approved by the Ethical committee of Stanley Medical College.

The detailed procedure and purpose of the study was explained in the regional language, and then an informed and written consent was obtained from the subjects if they were 16 years of age or over and from their parents if they were younger than 16 years.

EQUIPMENT FOR HRV

ECG was acquired using RMS Polyrite D hardware 2.2 (India), and instantaneous heart rate at RR intervals were plotted using RMS 2.5.2 software on a Microsoft window based PC. The RMS Polyrite 2.5.2 helps to save multiple records and provided with additional filter settings, calculation tools, automated analysis and auto report generation. Respiratory movements were recorded using respiratory belt.

METHODOLOGY OF HRV

The recordings were done between 10 a.m. and 12 noon to avoid circadian variations. Height and weight were taken. Blood Pressure was recorded using sphygmomanometer. The lab environment was quiet, the temperature was maintained between 25 to 28˚C and the lighting subdued. Subjects were asked to empty their bladder before the tests. The test did not involve any intravascular instrumentation or administration of any drugs at any stage.

The subjects were made to sit in the lab for 10 minutes to get accustomed to the new environment. The subjects were clearly instructed not to take coffee, tea or cool drinks 1½ hours before test.

1. Electrodes were fixed in the following position after cleaning with spirit to record the ECG

Electrode	Position
Exploring Electrode	Left shoulder/forearm
Exploring Electrode	Right shoulder/forearm
Reference Electrode	Right leg

2. Respiratory belt was tied around the chest at the level of the nipple to record respiratory movement

3. The electrodes and the respiratory belt were connected to RMS Polyrite D equipment

i) HRV during supine rest

ECG was recorded for 10 minutes to determine the HRV at supine rest with the eyes closed with normal quiet respiratory movement.

ii) HRV response to standing

After recording in the supine position the subject was asked to stand without support on a wooden plank and ECG was recorded.

iii) HRV during one minute controlled deep breathing

After recording in the standing position the subject was asked to lie down comfortably in the supine position. He was then instructed to breathe slowly and deeply at the rate of 6 breaths per minute in such a way that he takes 5 seconds for inspiration and 5 seconds for expiration. The entire procedure was monitored on the screen. Deep breathing will produce maximum Respiratory Sinus Arrhythmia.

After 10 minutes of rest, Visual acuity was checked using Snellen's chart. Then, Visual Evoked Potential (VEP) was recorded.

METHODOLOGY OF V.E.P.

By using the standard RMS EMG EP MARK II machine VEP recordings were done by using standard procedures.

PRE –REQUISITES:

1. Avoid hair spray or oil after hair wash.

2. If subject has refractory error, the usual glasses are put on during the test.

3. Any miotic or mydriatic drug is avoided 12 hours before the test.

EQUIPMENT SET UP FOR VEP STUDY:

MONTAGE:

Channel 1 - FPz – Reference electrode.

Vertex - Cz – Ground electrode.

C - Oz - Active electrode.

RECORDING CONDITIONS:

1. Filter: high filter cut: 100 – 300 Hz.

2. Amplification between 20,000 – 1,00,000

3. Sweep Duration: 300 msecs.

4. Number of epochs: 100 are averaged.

5. Electrode impedance kept below 5 Kilo ohms.

STIMULATION:

Black and white Checkerboard is used. Distance between subject and screen was 100 cms.

Contrast: 80%

Size of the pattern element: 14x16 minute

Rate of stimulation: 4 – 8 Hz.

PROCEDURE:

The subject was asked to sit comfortably on a chair with their footwear.

Each eye was tested separately.

The other eye was kept covered with an opaque eye shield, which prevents entry of light into that eye. The skin at the point of placement of electrodes was cleansed with spirit.

Three surface disc type electrodes are used.

Using electrode paste, the **recording electrode** is placed at Oz – 5cm above the inion (ridge between the back of neck & skull).

The **reference electrode** is placed at FPz – 12cms above the nasion (indentation between forehead and nose).

The **ground electrode** is placed at the midline in forehead.

The electrodes are connected through the pre amplifier to the Cathode ray Oscilloscope.

The subject is instructed to fix the gaze at the centre of the screen.

The lights are switched off.

The visual stimulus is delivered by photo stimulator at frequency of 10 flashes/sec.

The response obtained is displayed on the T V monitor and the peak latency and peak to peak amplitude of the waves are measured.

NORMAL VEP:

VEP consists of series of wave forms of opposite polarity, the negative wave form (denoted as N) & a positive wave form (denoted as P), which is followed by the approximate latency in millisecond.

OBSERVATIONS

TABLE 1

ANTHROPOMETRIC MEASUREMENTS OF SUBJECTS

(Age, Height, Weight & BMI expressed as Mean ± SD)

	CASES	**CONTROLS**	**'t'value**	**p value**
n	30	30	-	-
Males:Females	18:12	18:12	-	-
Age in years	23.06 ± 6.06	22.86 ± 5.92	0.12	0.89
Height in cms.	155.96 ± 9.25	156.80 ± 9.57	-0.34	0.73
Weight in kg.	53.83 ± 7.86	54.23 ± 7.88	-0.19	0.84
B.M.I. kg/m2	22.07 ± 2.31	21.98 ± 2.17	0.15	0.87

BMI – Body Mass Index

The parameters were analyzed using Student independent unpaired 't'test.

$p < 0.05$ is taken as significant

TABLE 2

HEART RATE & BLOOD PRESSURE

MEASUREMENTS

	GROUP (n=30)	**MEAN**	**STANDARD DEVIATION**	**Student independent 't' test**
Resting Heart rate In bpm	Cases	87.3	8.4	t = 4.05 **p < 0.01****
	Controls	76.4	12.01	
Systolic B.P. (mm.Hg.)	Cases	120.60	5.61	t=1.597 p=0.116
	Controls	118.00	6.92	
Diastolic B.P. (mm.Hg).	Cases	79.40	4.64	t=0.858 p=0.395
	Controls	78.33	4.98	

The parameters were analyzed using Student independent unpaired 't'test.

$p < 0.05$* is taken as significant ;$p < 0.01$** is taken as highly significant.

TABLE 3

COMPARISON OF BLOOD SUGAR LEVEL

	GROUP (n=30)	**MEAN**	**STANDARD DEVIATION**	**Student independent 't' test**
Fasting mg%	Cases	124.06	18.20	t= 12.22 **p< 0.01****
	Controls	82.33	4.29	
Post prandial mg%	Cases	158.34	31.22	t=8.77 **p<0.01****
	Controls	107.93	3.94	

The parameters were analyzed using Student independent unpaired 't'test.

$p < 0.05$* is taken as significant ;$p < 0.01$** is taken as highly significant.

FIGURE - 1

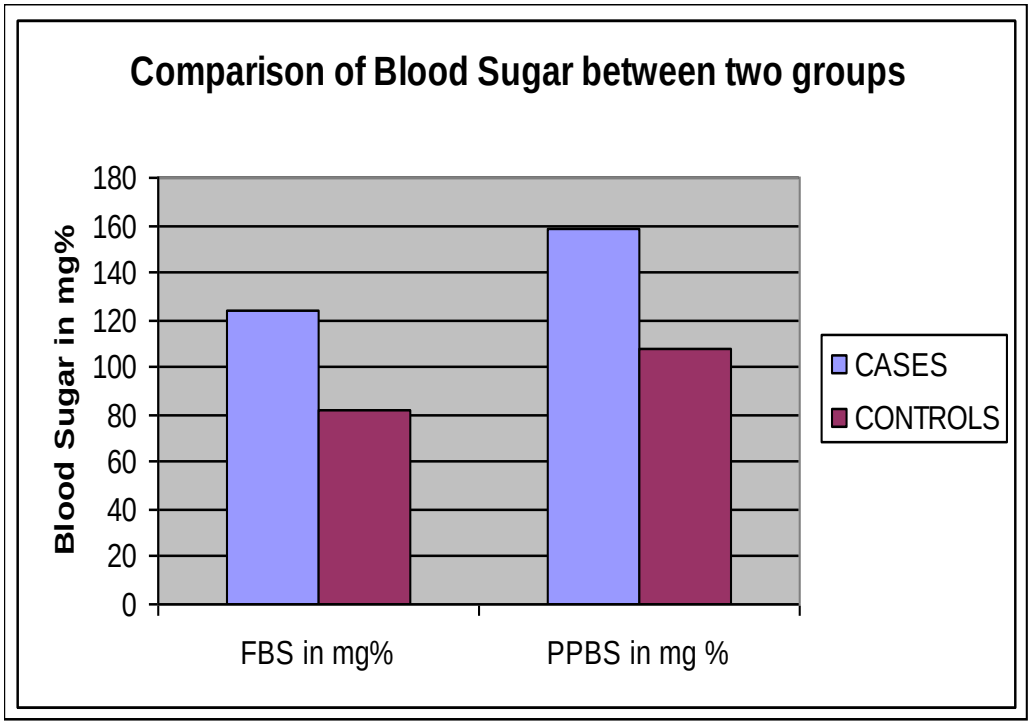

Comparison of Blood Sugar between two groups
Blood Sugar in mg%
180
160
140
120
100
80
60
40
20
0
FBS in mg%
PPBS in mg %
CASES
CONTROLS

TABLE 4

CHANGES IN FREQUENCY DOMAIN MEASURES DURING SUPINE REST

Frequency Domain Measures	**Cases (n=30)**		**Controls (n=30)**		**Student independent 't' test**
	Mean	SD	Mean	SD	
Mean RR in sec.	0.69	0.07	0.80	0.12	t=-4.019 **p< 0.01****
LF ms^2	23.21	13.99	15.18	12.45	t= 2.34 **p< 0.05***
HF ms^2	7.7	6.0	17.22	14.84	t= -3.25 **p< 0.01****
LF/HF	3.46	1.88	1.16	0.75	t=6.20 **p< 0.01****
TOTAL POWER ms^2	30.91	18.69	32.40	23.70	t=-0.27 p=0.788
LF n.u.	74.29	8.80	49.11	14.97	t= 7.94 **p< 0.01****
HF n.u.	25.50	8.79	50.87	14.91	t= - 8.02 **p< 0.01****

$p < 0.05$* is taken as significant ;$p < 0.01$** is taken as highly significant

TABLE 5

CHANGES IN TIME DOMAIN MEASURES DURING SUPINE REST

Time Domain Measures	**Cases (n=30)**		**Controls (n=30)**		**Student independent 't' test**
	Mean	SD	Mean	SD	
SDNN in ms	28.54	13.45	56.61	23.42	t= - 5.69 **p<0.01****
RMSSD in ms	24.48	12.73	68.57	28.04	t=- 7.84 **p<0.01****
NN50 count	9.73	14.48	116.83	74.26	t= - 7.75 **p<0.01****
pNN50%	2.42	3.84	33.55	23.56	t= - 7.14 **p<0.01****

The parameters were analyzed using Student independent unpaired 't'test.

$p < 0.05$* is taken as significant ;$p < 0.01$** is taken as highly significant

FIGURE - 2

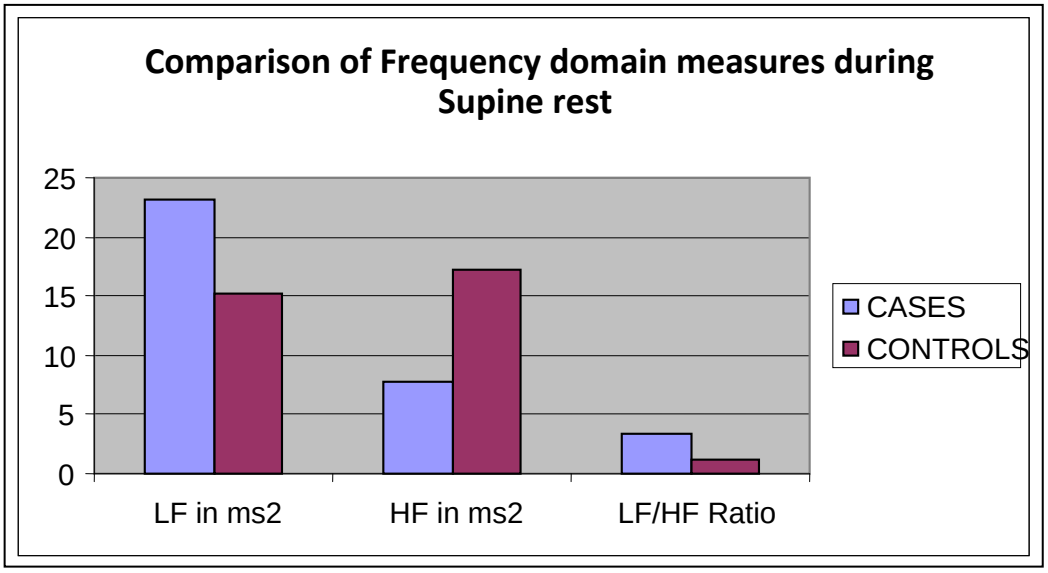


FIGURE - 3

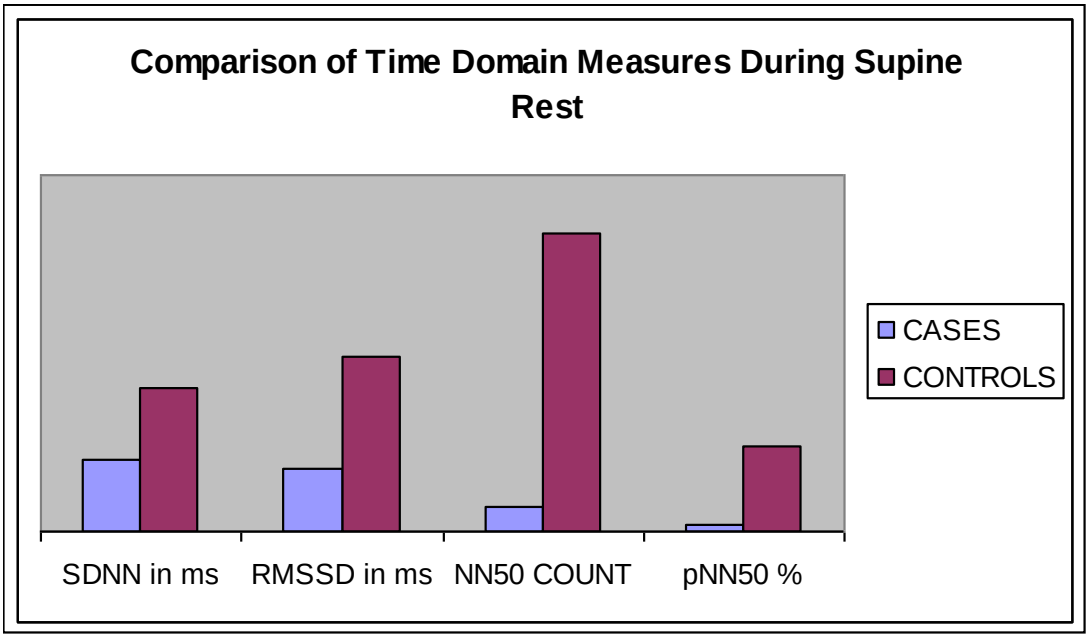


TABLE 6

CHANGES IN FREQUENCY DOMAIN MEASURES DURING STANDING

Frequency Domain Measures	**Cases (n=30)**		**Controls (n=30)**		**Student independent 't' test**
	Mean	SD	Mean	SD	
Mean RR in sec.	0.60	0.05	0.69	0.10	t=-4.045 **p<0.01****
LF ms^2	45.91	18.97	29.08	18.05	t= 3.52 **p<0.01****
HF ms^2	8.72	5.18	14.29	10.82	t= - 2.54 **p<0.05***
LF/HF	6.50	3.54	3.17	2.74	t= 4.06 **p<0.01****
TOTAL POWER ms^2	54.63	20.43	43.37	24.47	t=1.935 p=0.058
LF n.u.	83.29	9.03	68.14	14.05	t= 4.96 **p< 0.01****
HF n.u.	16.72	9.04	31.93	14.02	t= - 4.992 **p< 0.01****

$p < 0.05$* is taken as significant; $p < 0.01$** is taken as highly significant

TABLE 7

CHANGES IN TIME DOMAIN MEASURES DURING STANDING

Time Domain Measures	**Cases (n=30)**		**Controls (n=30)**		**Student independent 't' test**
	Mean	SD	Mean	SD	
SDNN ms	27.99	11.33	49.81	20.16	t= - 5.16 **p<0.01****
RMSSD ms	20.13	11.25	41.40	22.09	t= - 4.7 **p<0.01****
NN50	7.93	14.80	54.66	67.30	t= - 3.71 **p < 0.01****
pNN50%	1.62	3.00	14.23	19.00	t= - 3.58 **p<0.01****

The parameters were analyzed using Student independent unpaired 't'test.

$p < 0.05$* is taken as significant ;$p < 0.01$** is taken as highly significant

FIGURE - 4

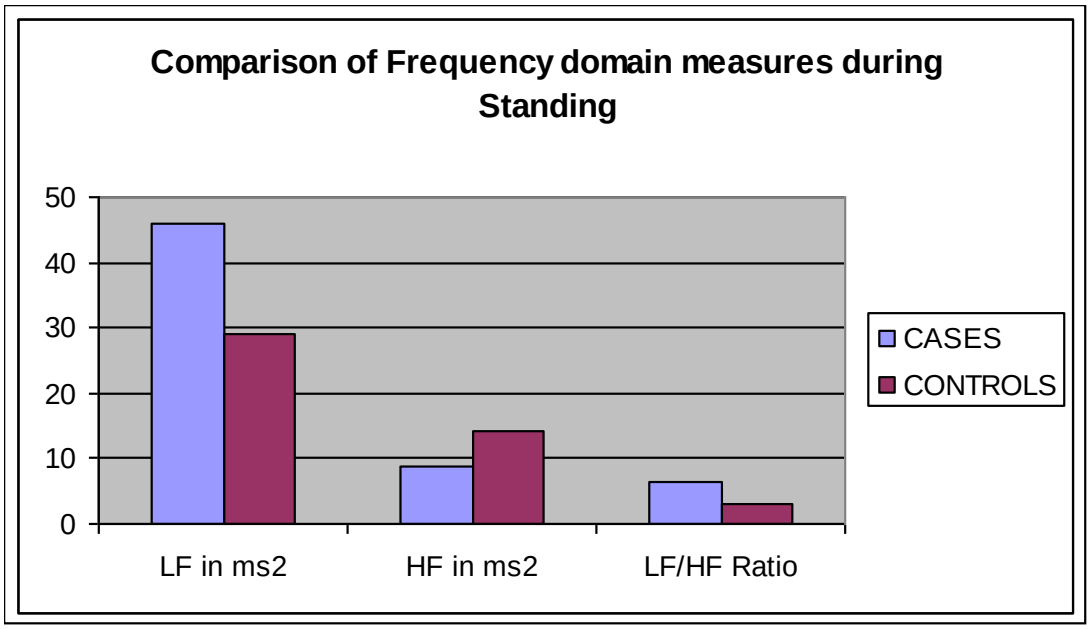


FIGURE - 5

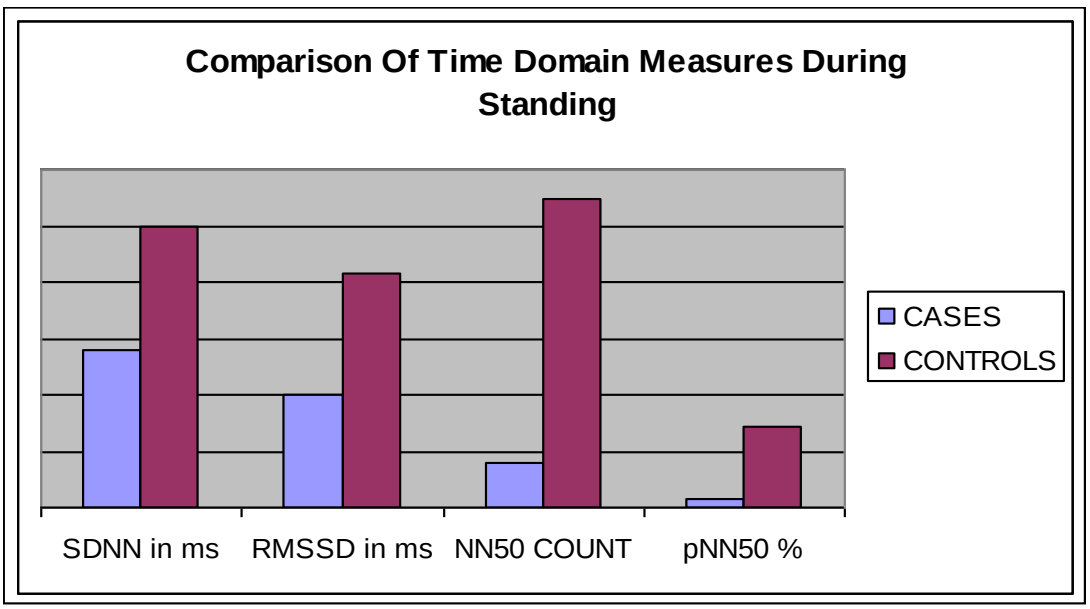


TABLE 8

FREQUENCY DOMAIN MEASURES DURING ONE MINUTE DEEP BREATHING

Frequency Domain Measures	**Cases (n=30)**		**Controls (n=30)**		**Student independent 't' test**
	Mean	SD	Mean	SD	
Mean RR in sec.	0.72	0.07	0.83	0.11	t= -4.091 **p<0.01****
HF in ms^2	0.82	1.01	1.91	1.72	t= - 3.008 **p<0.01****
HF in n.u.	18.89	9.10	28.40	11.89	t= - 3.47 **p<0.01****

The parameters were analyzed using Student independent unpaired 't'test.

$p < 0.05$* is taken as significant ;$p < 0.01$** is taken as highly significant

TABLE 9

TIME DOMAIN MEASURES DURING ONE MINUTE DEEP BREATHING

Time Domain Measures	**Cases (n=30)**		**Controls (n=30)**		**Student independent 't' test**
	Mean	SD	Mean	SD	
SDNN ms	50.33	19.17	88.76	20.56	t= - 7.48 **p<0.01****
RMSSD ms	56.62	32.12	98.76	24.18	t= - 5.74 **p<0.01****
NN50	8.73	7.82	28.16	11.56	t= - 7.62 **p<0.01****
pNN50%	14.25	14.43	38.72	15.76	t= - 6.27 **p< 0.01****

The parameters were analyzed using Student independent unpaired 't'test.

$p < 0.05$* is taken as significant ;$p < 0.01$** is taken as highly significant

FIGURE - 6

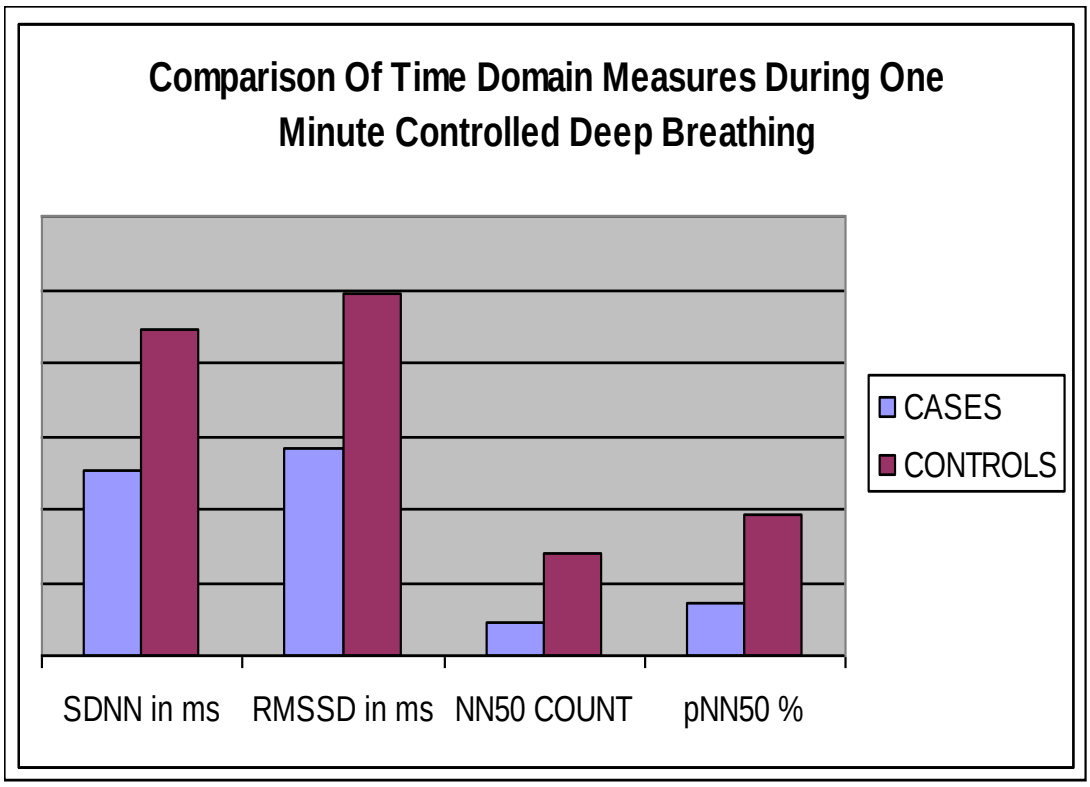

Comparison Of Time Domain Measures During One Minute Controlled Deep Breathing
CASES
CONTROLS
SDNN in ms
RMSSD in ms
NN50 COUNT
pNN50 %

TABLE 10

VISUAL EVOKED POTENTIALS RECORDED FROM LEFT EYE

VEP parameters	**Cases (n=30)**		**Controls (n=30)**		**Student independent 't'test**
	Mean	SD	Mean	SD	
N 75 ms	68.97	4.73	66.71	3.31	t= 2.14 **p<0.05***
P100 ms	96.87	5.48	89.52	2.56	t= 6.64 **p < 0.01****
N145 ms	147.05	9.78	147.18	9.21	t= - 0.052 p= 0.95
P 100 - N 75 µV	5.88	2.43	6.94	1.37	t= - 2.04 **p<0.05***

The parameters were analyzed using Student independent unpaired 't'test.

p < 0.05* is taken as significant ;p < 0.01** is taken as highly significant

TABLE 11

VISUAL EVOKED POTENTIALS RECORDED FROM RIGHT EYE

VEP parameters	**Cases (n=30)**		**Controls (n=30)**		**Student independent 't'test**
	Mean	SD	Mean	SD	
N 75 ms	69.77	5.05	67.37	2.55	t= 2.32 **p<0.05***
P100 ms	96.91	6.03	89.87	2.79	t= 5.80 **p < 0.01****
N145 ms	146.27	9.25	146.65	9.41	t= - 0.15 p= 0.87
P 100 - N 75 µV	5.88	2.06	7.01	1.81	t= - 2.25 **p < 0.05***

The parameters were analyzed using Student independent unpaired 't'test.

p < 0.05* is taken as significant ;p < 0.01** is taken as highly significant

FIGURE - 7

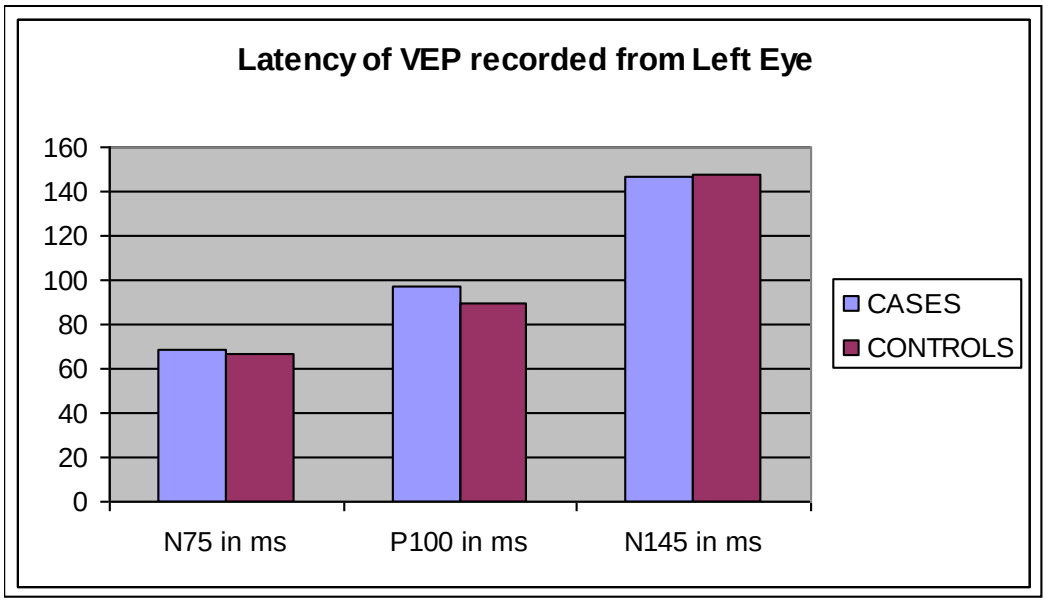


FIGURE - 8

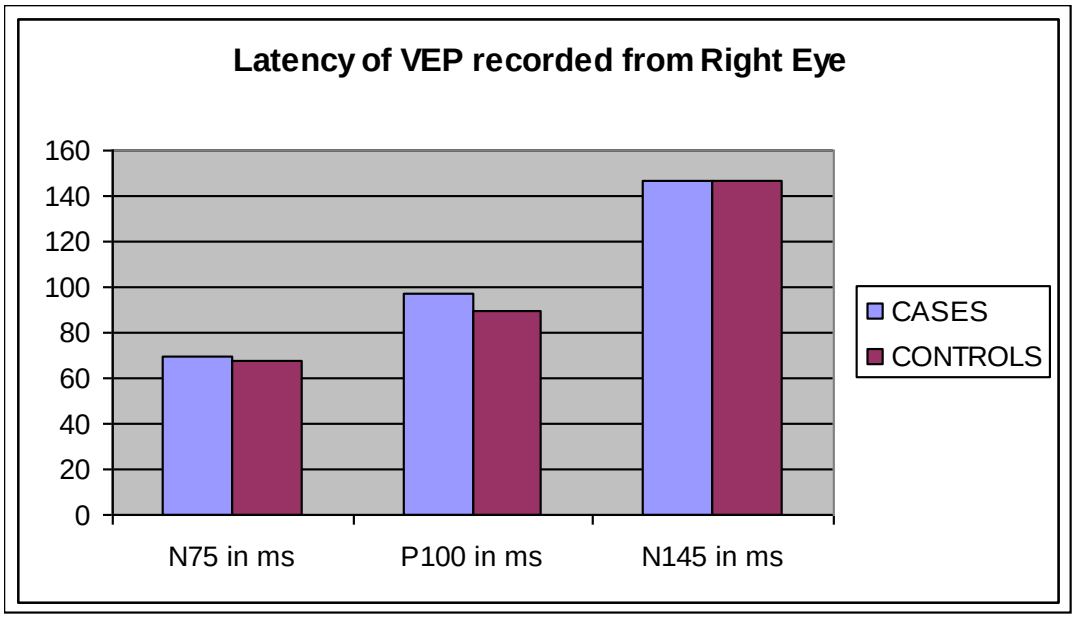


FIGURE - 9

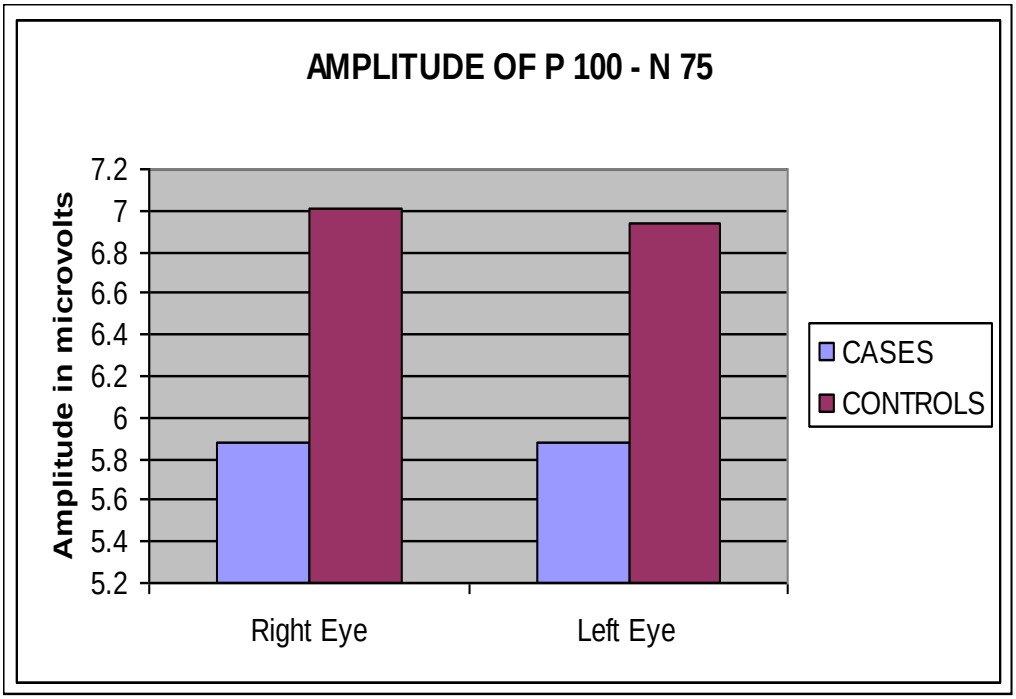

AMPLITUDE OF P 100 - N 75
Amplitude in microvolts
7.2
7
6.8
6.6
6.4
6.2
6
5.8
5.6
5.4
5.2
Right Eye
Left Eye
CASES
CONTROLS

TABLE 12

CORRELATION BETWEEN P100 & HRV INDICES

USING PEARSON'S CORRELATION COEFFICIENT

	CASES	**CONTROLS**
SDNN	-0.187 p=0.323	0.216 p=0.252
LF	-0.273 p=0.144	-0.175 p=0.355
HF	-0.186 p=0.324	-0.026 p=0.893
LF/HF	0.080 p=0.673	-0.067 p=0.726

****** Correlation is significant at the 0.01 level (2 tailed)

* Correlation is significant at the 0.05 level (2 tailed)

RESULTS

Statistical Package for Social Sciences (SPSS) software 11.5 version was used for statistical analysis. The Student independent unpaired 't' test was used to compare cases and controls. (Tables 1- 11).

Pearson's correlation test was used to correlate between VEP and HRV parameters (Table 12).

TABLE 1: ANTHROPOMETRIC MEASUREMENTS OF SUBJECTS

There was no statistical difference between the cases and controls with regards to age, height, weight and BMI ($p > 0.05$).

TABLE 2: HEART RATE & BLOOD PRESSURE MEASUREMENTS

There was no statistical difference between the cases and controls with regards to SBP & DBP ($p > 0.05$). There was highly significant increase in resting Heart Rate in the cases. ($p < 0.01$)

TABLE 3: BLOOD SUGAR:

There was a highly significant increase in fasting and post-prandial blood sugar in cases compared to controls. ($p < 0.01$)

TABLE 4: CHANGES IN FREQUENCY DOMAIN MEASURES DURING SUPINE REST

LF in absolute power was significantly increased in cases, LF nu was highly significantly increased in cases. HF in absolute power as well as in normalized units was highly significantly reduced in cases. LF/HF was highly significantly increased in cases.

TABLE 5: CHANGES IN TIME DOMAIN MEASURES DURING SUPINE REST

The SDNN, RMSSD, NN50 and pNN50 was highly significantly reduced in cases as compared to controls ($p < 0.01$)

TABLE 6: CHANGES IN FREQUENCY DOMAIN MEASURES DURING STANDING

LF power and LFnu was significantly increased in cases. HF power was significantly reduced in cases. HFnu was highly significantly reduced in cases. LF/HF was highly significantly increased in cases.

TABLE 7: CHANGES IN TIME DOMAIN MEASURES DURING STANDING

The SDNN, RMSSD, NN50 & pNN50 was highly significantly reduced in cases during standing($p<0.01$)

TABLE 8: CHANGES IN FREQUENCY DOMAIN MEASURES DURING ONE MINUTE DEEP BREATHING

HF power and HFnu was highly significantly reduced in cases.($p <0.01$)

TABLE 9: CHANGES IN TIME DOMAIN MEASURES DURING ONE MINUTE DEEP BREATHING

SDNN, RMSSD, NN50, pNN50 was highly significantly reduced in cases compared to controls($p<0.01$),during one minute deep breathing

TABLE 10: VISUAL EVOKED POTENTIALS RECORDED FROM LEFT EYE

N75 latency in the left eye was significantly increased in cases ($p < 0.05$). There was a highly significant increase in P100 latency of the left eye in the cases compared to controls($p < 0.01$).

The amplitude of P100-N75 of the left eye was significantly reduced in cases compared to controls($p < 0.05$)

TABLE 11: VISUAL EVOKED POTENTIALS RECORDED FROM RIGHT EYE

The latency of N75 was significantly increased in cases ($p < 0.05$)

There was a highly significant increase in P100 latency of the right eye in cases compared to controls ($p < 0.01$)

The amplitude of P100-N75 of the right eye was significantly reduced in cases compared to controls($p < 0.05$)

TABLE 12: CORRELATION BETWEEN P100 & HRV INDICES

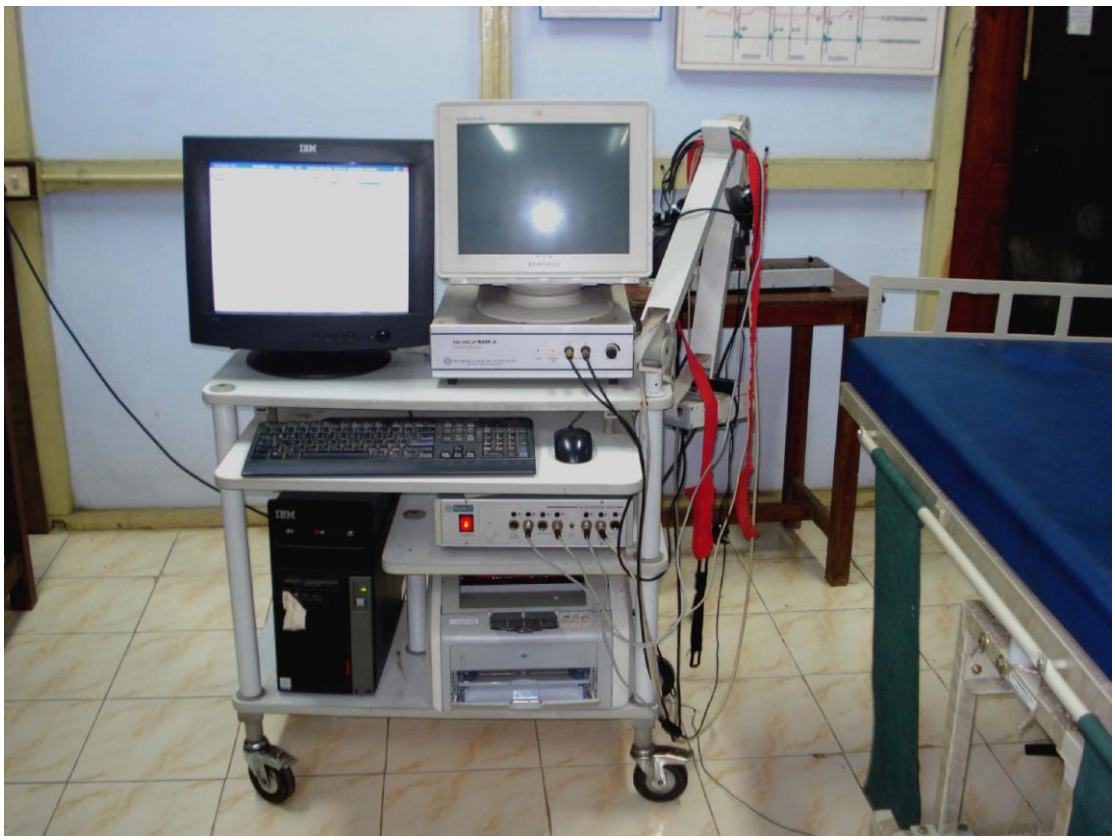
PEARSON'S CORRELATION TEST

There was a negative correlation between P100 and SDNN in cases but it was not significant.

LF and HF power had a negative correlation with P100, which was not statistically significant.

There was a non-significant positive correlation between P100 and LF/HF in cases.

RMS POLYRITE



HRV RECORDING OF A SUBJECT DURING SUPINE REST

HRV RECORDING OF A SUBJECT DURING STANDING

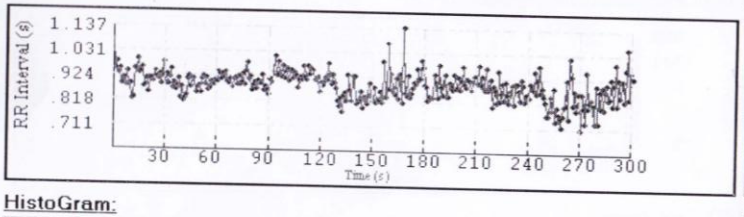
RECORDING OF VISUAL EVOKED POTENTIALS

Heart Rate Variability Analysis

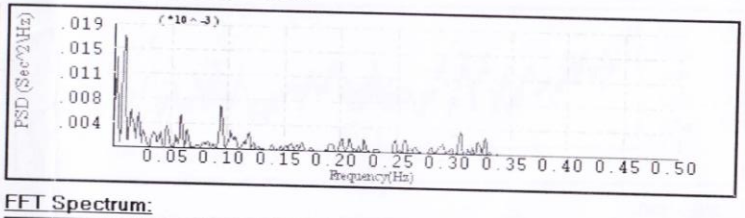
Subject : [redacted] Id : s011

SUPINE REST

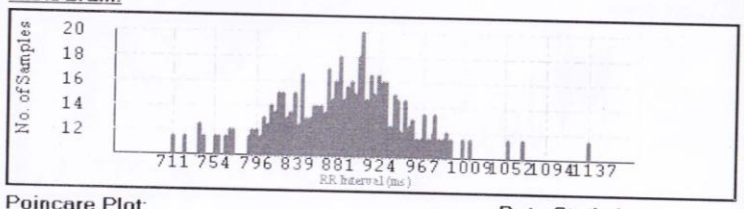
RR TachoGram:



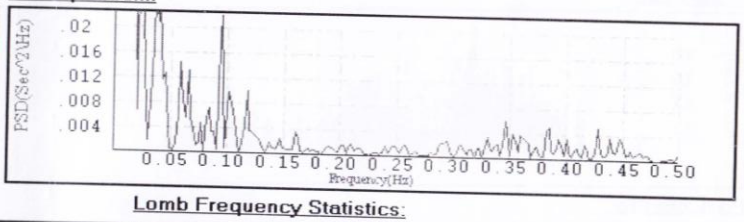
Lomb Periodogram Spectrum:



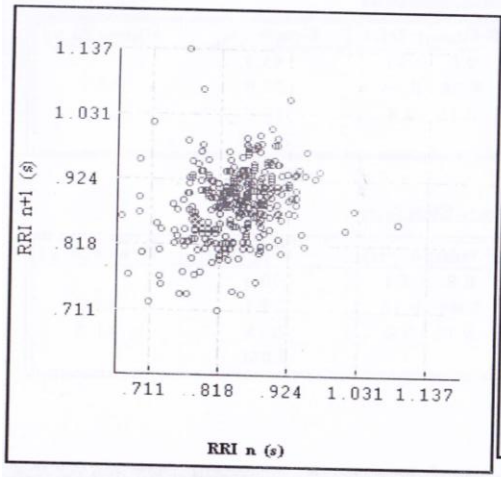
HistoGram:



FFT Spectrum:



Poincare Plot:



Data Statistics:

Parameter	Value
Max RR	1.137 s
Min RR	0.711 s
Max/Min	1.599
Mean RR	0.884 s
Max HR	84 bpm
Min HR	53 bpm
Mean HR	68 bpm

Parameter	Value
SDNN	56.808 ms
RMSSD	85.666
NN50	141 count
pNN50	41.6 %
Variance	3227.14 ms^2
Sample Points	339
Range	301

Lomb Frequency Statistics:

	F-Range (Hz)	Power (%)	Power (n.u.)
VLF	0.0 - 0.04	42.0	
LF	0.04 - 0.15	29.7	51.2
HF	0.15 - 0.4	28.4	49.
LF/HF		1.046	

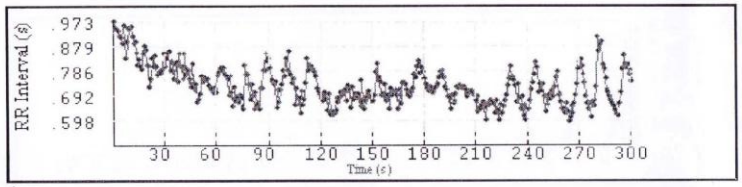
FFT Frequency Statistics:

	F-Range (Hz)	Power (%)	Power (n.u.)
VLF	0.0 - 0.04	81.4	
LF	0.04 - 0.15	11.9	64.
HF	0.15 - 0.4	06.7	36.
LF/HF		1.776	

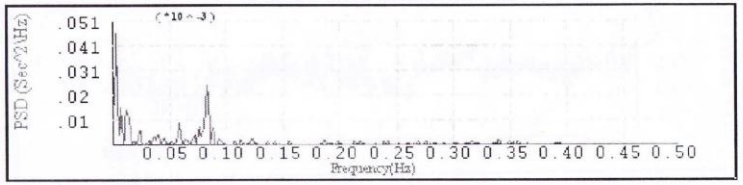
Heart Rate Variability Analysis

Subject : [redacted] **Id :** s011 STANDING

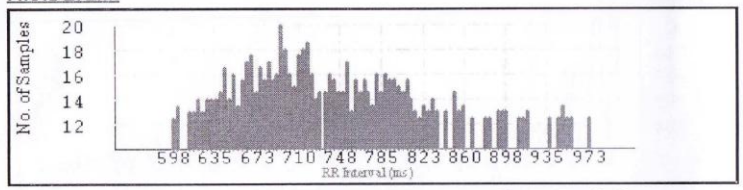
RR TachoGram:



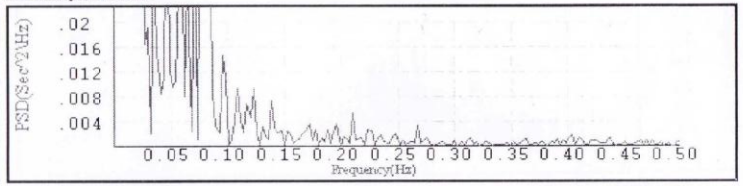
Lomb Periodogram Spectrum:



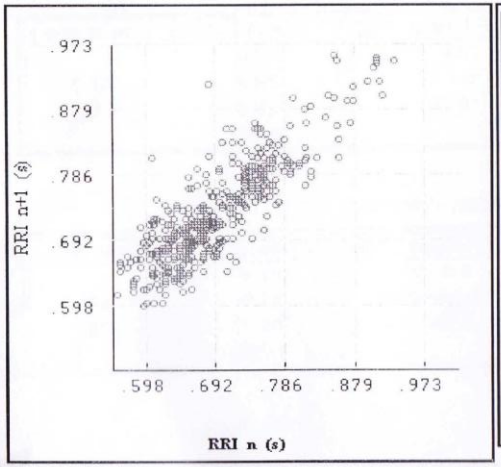
HistoGram:



FFT Spectrum:



Poincare Plot:



Data Statistics:

Parameter	Value
Max RR	0.973 s
Min RR	0.598 s
Max/Min	1.627
Mean RR	0.733 s
Max HR	100 bpm
Min HR	62 bpm
Mean HR	82 bpm
Parameter	**Value**
SDNN	73.643 ms
RMSSD	40.473
NN50	88 count
pNN50	22.2 %
Variance	5423.31 ms^2
Sample Points	396
Range	292

Lomb Frequency Statistics:

	F-Range (Hz)	Power (%)	Power (n.u.)
VLF	0.0 - 0.04	43.9	
LF	0.04 - 0.15	38.0	67.7
HF	0.15 - 0.4	18.2	32.4
LF/HF		2.088	

FFT Frequency Statistics:

	F-Range (Hz)	Power (%)	Power (n.u.)
VLF	0.0 - 0.04	68.3	
LF	0.04 - 0.15	28.1	88.6
HF	0.15 - 0.4	03.6	11.4
LF/HF		7.806	

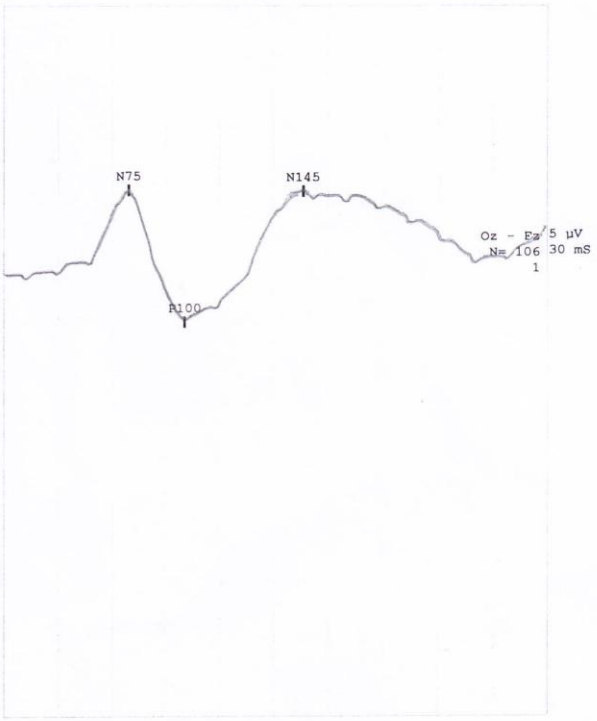
RMS ADVANCE TESTING LAB

181/5 Phase I Industrial Area Chandigarh
Phones 658701-705

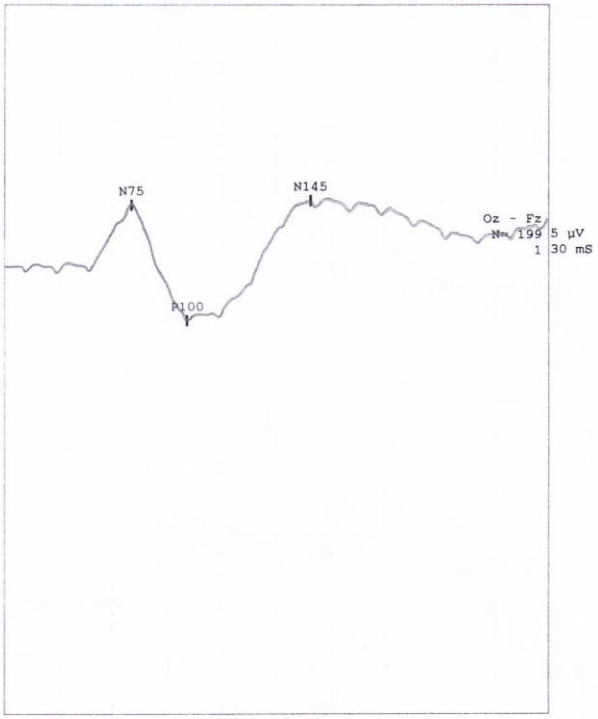
J005 [redacted] | 10/0 Yrs/Mths Female | 135 Cms/30 Kg
Physician: Dr. [redacted] | Ref By: | Date: 17-Aug-2010

VEP RECORD

LEFT



RIGHT



LEFT

Tr	Montage	N75 (mS)	P100 (mS)	N145 (mS)	(mS)	(mS)	P100 - N75 (µV)
1	Oz - Fz	69.4	100.6	166.3			12.16
2	Oz - Fz						

RIGHT

Tr	Montage	N75 (mS)	P100 (mS)	N145 (mS)	(mS)	(mS)	P100 - N75 (µV)
1	Oz - Fz	70.0	100.6	168.1			10.63
2	Oz - Fz						

Test Comments

NOTE:THE RESULTS MAY BE CLINICALLY CORRELATED.

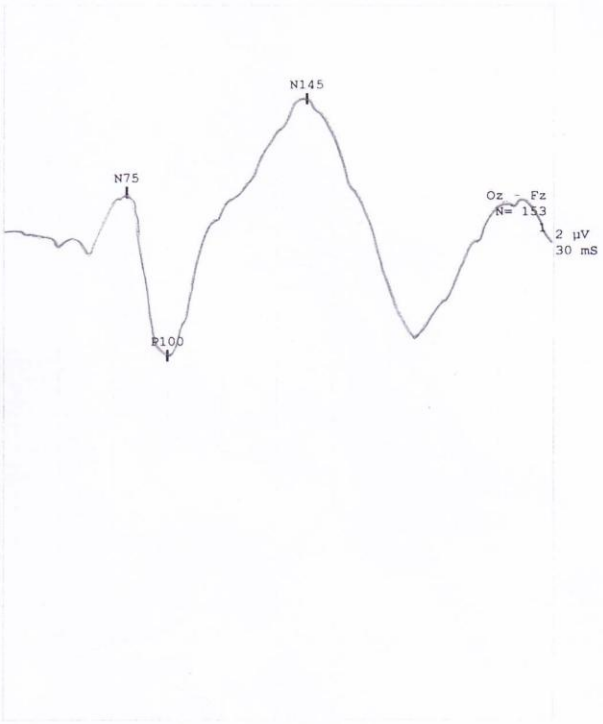
RMS ADVANCE TESTING LAB

181/5 Phase I Industrial Area Chandigarh
Phones 658701-705

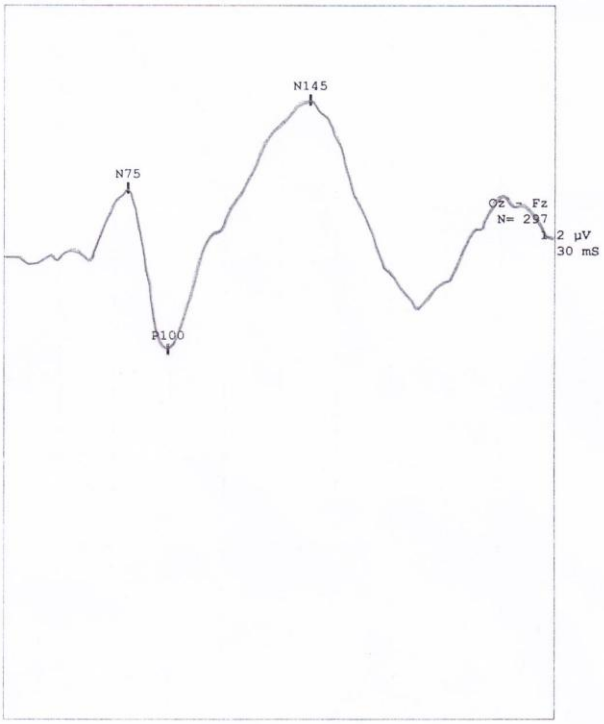
S007 [redacted] | 17/0 Yrs/Mths Female | 155 Cms/53 Kg
Physician: Dr [redacted] | Ref By: | Date: 15-Sep-2010

VEP RECORD

LEFT



RIGHT



LEFT

Tr	Montage	N75 (mS)	P100 (mS)	N145 (mS)	(mS)	(mS)	P100 - N75 (µV)
1	Oz - Fz	67.5	90.0	166.3			5.87
2	Oz - Fz						

RIGHT

Tr	Montage	N75 (mS)	P100 (mS)	N145 (mS)	(mS)	(mS)	P100 - N75 (µV)
1	Oz - Fz	67.5	89.4	166.3			5.85
2	Oz - Fz						

Test Comments

NOTE:THE RESULTS MAY BE CLINICALLY CORRELATED.

DISCUSSION

In type 1 diabetes, Cardiovascular autonomic neuropathy (CAN) is ultimately the result of complex interactions among degree of glycemic control, disease duration and systolic and diastolic blood pressure (Witte DR et al 2005)[60]. Hyperglycemia plays the key role in the activation of various biochemical pathways related to the metabolic and/or redox state of the cell, which, in concert with impaired nerve perfusion, contribute to the development and progression of diabetic neuropathies. Experimental data implicate a number of pathogenic pathways that may impact autonomic neuronal function in diabetes including: formation of advanced glycation end products, increased oxidative/nitrosative stress with increased free radical production, activation of the polyol and protein kinase C pathways, activation of polyADP ribosylation, and activation of genes involved in neuronal damage (Vinik AI et al 2003) [26].

CAN AND CARDIAC DYSFUNCTION

Autonomic innervation is the primary extrinsic control mechanism regulating HRV and cardiac performance. It has been shown that chronic hyperglycemia promotes progressive autonomic neural dysfunction. The vagus nerve, the longest autonomic nerve, mediates 75% of all parasympathetic activity (Ziegler D et al 1992) [61]. Because neuropathy is seen first in the longest fibers, the earliest manifestation of autonomic neuropathy in diabetes tends to be associated with parasympathetic denervation [18,25]. Rodica Pop-Busui et al

(2010) [62], confirm that early in the progression of CAN complicating type 1 diabetes, there is a compensatory increase in the cardiac sympathetic tone.

Clinical symptoms of autonomic dysfunction may not appear until long after diabetes onset. However, subclinical CAN, manifested as changes in HRV, may be detected within 1 year of diagnosis in type 2 diabetes and within 2 years of diagnosis in type 1 diabetes (Pfeifer et al 1984) [63].

IMPAIRED HRV

Variability in the instantaneous beat-to-beat heart rate intervals is a function of sympathetic and parasympathetic activity that regulates the cardiac functional response to the body's level of metabolic activity. In normal individuals the heart rate has a high degree of beat to- beat variability and HRV fluctuates with respiration—increasing with inspiration and decreasing with expiration. The most frequent finding in subclinical CAN is reduced heart rate variability (Ziegler D et al 1994) [64]

HRV IN SUPINE REST:

The Resting heart rate was significantly increased in the Type 1 diabetic patients compared to controls ($p<0.01$**). This resting tachycardia is due to vagal impairment associated with a relative increase in the sympathetic tone. This is similar to the findings observed in the review by Vinik AI et al (2003) [26].

FREQUENCY DOMAIN MEASURES:

Mechanisms influencing heart period modulation can be assumed to be stationary in short term recordings (Task force report, 1996) [4]. HRV occurring in the frequency range 40 – 400 mHz is mainly due to two mechanisms – tonic vagal activity and reflex vagal activity. It is important to appreciate that power in this range signifies the extent to which HR is modulated by acetylcholine released upon stimulation of cardiac vagal nerves. It is an index of parasympathetic modulation of instantaneous heart rate and is also dependent upon the sensitivity of effectors to acetylcholine. It does not quantify and may not correlate with vagal nerve traffic. Vagal tone is best quantified by the change in heart rate induced by atropine after total beta-blockade (Stein PK, 1999) [65].

The efferent vagal activity is a major contributor of the HF component as seen in clinical and experimental observations of autonomic maneuvers such as electrical vagal stimulation, muscarinic receptor blockade and vagotomy (Akselrod et al 1981) [13]. In accordance basal High frequency power is significantly reduced in the diabetics compared to controls ($p<0.01$**). HF nu which represents parasympathetic activity was significantly lower in the diabetic group.

The LF component is considered by some as a marker of sympathetic modulation, especially when expressing it as normalized units and by others as a

parameter that includes both sympathetic and parasympathetic influences. LF power was significantly increased in diabetics compared to controls ($p<0.05$*). LFnu was highly significantly increased in diabetics ($p<0.01$**).

LF/HF ratio is considered to mirror sympatho-vagal balance(Malik M et al 1993) [66]. LF/HF was significantly increased in the diabetic group($p<0.01$**). Type 1 diabetics exhibit altered sympatho-vagal balance with decreased parasympathetic activity at the cardiac level. Similar changes in HRV parameters were observed by Massimo Chessa et al (2002) [25].

TIME DOMAIN INDICES:

The variations in heart rate may be evaluated by a number of methods. Perhaps the simplest to perform are the time domain measures. The simplest variable to calculate is the standard deviation of the NN intervals (SDNN) that is, the square root of variance. Since variance is mathematically equal to total power of spectral analysis, SDNN reflects all the cyclic components responsible for variability in the period of recording. The SDNN was significantly decreased in type 1 diabetics ($p < 0.01$**).The findings are in accordance with the results of Dariusz Korczak et al (1997) [18].

In our observations, RMSSD and pNN50 was significantly reduced

($p < 0.01$**). NN50 count was also reduced significantly in the cases ($p < 0.01$**).All these observations are a pointer towards a reduced parasympathetic activity in diabetics.

HRV IN STANDING:

FREQUENCY DOMAIN MEASURES:

The LF in absolute power was highly significantly increased in diabetics compared to the controls ($p<0.01$**). The HF in absolute power was significantly reduced in diabetics. The LF/HF ratio showed a highly significant increase in the diabetic group. LF nu was significantly increased in diabetics and HF nu was significantly reduced.

TIME DOMAIN INDICES:

SDNN was highly significantly reduced($p < 0.01$**) in diabetics compared to controls.. RMSSD , NN50 & pNN50 was also highly significantly reduced in diabetics after standing when compared to controls. Our findings are consistent with the results of Massimo Chessa et al (2002) [25].

HRV in DEEP BREATHING:

Respiratory sinus arrhythmia (RSA) is a change of heart rate generated by a combination of respiration-induced biochemical changes, changes in intrathoracic pressure, and central vagal stimulation.(P. Zhang et al 1997)[67]. Respiration has a significant effect on the HR oscillations and parasympathetic activity is very closely related to respiratory sinus rhythm. Frequency of RSA component falls in High Frequency range of HRV (0.15-0.4 Hz) for more than 6

breaths/min. On one minute controlled deep breathing at 6 breaths/min, with inspiration and expiration each lasting for 5 secs. the mean RR was significantly reduced in diabetic patients(p<0.01**). In the frequency domain measures the HF in absolute power and HF in normalized units was significantly reduced in diabetics (p<0.01**) which might have been due to vagal neuropathy [18,25]. HRV during timed deep breathing is a major index of HR variation in the time domain because it has been shown to be one of the most reliable and reproducible markers of parasympathetic modulation of cardiac function (Ewing et al 1985) [11]. All the time domain variables were significantly reduced in diabetics (p< 0.01**). Reduced HRV during deep breathing in type 1 diabetics clearly indicates that cardiac vagal effects are diminished in this condition. This may possibly be due to vagal neuropathy.

TYPE 1 DM AND CENTRAL NERVOUS SYSTEM ALTERATIONS:

Peripheral and Autonomic nervous system involvements are frequently encountered in DM, but there exists few data about the incidence of central diabetic neuropathies. It is possible to reveal central nervous system involvement at an early stage by using evoked potentials.(Uzun N et al 2006) [58]

Comi G et al (1987) [40], found that Visual Evoked Potentials is a simple and reliable technique for detecting early alterations in CNS function in diabetics.

VISUAL EVOKED POTENTIALS

N 75

The latency of N75 in right and left eye was significantly increased in diabetics compared to controls ($p<0.05$*)

P 100

There was a highly significant increase in P100 latency ($p< 0.01$**) in diabetics compared to controls

N 145

There was no significant difference in the latency of N145 in diabetic patients compared to controls.

Alessandrini M et al (1999) [68], observed a significant delay in N75, P100 and N145 latencies. But in our study only N75 and P100 were significantly prolonged.

P100- N 75

The amplitude of P100- N 75 was significantly reduced in both eyes in cases ($p< 0.05$*)

Karlica D et al (2010)[59] found that amplitude values decrease progressively and latency values increase progressively in children with DM 1 as the years pass.

Our results clearly indicate that there is a significant neuronal visual loss involving the optic pathway which precedes the ophthalmoscopically detectable retinopathy in patients with type 1 DM.

CORRELATION OF VEP & HRV PARAMETERS

Tamas T. Varkonyi et al (2002) [55], recorded VEP and also performed the five standard cardiovascular autonomic function tests. He found a significant positive correlation between autonomic score and latency of P100.

Ewing's battery of tests are cumbersome, time consuming and not very sensitive (Martial M. Massin et al 1999) [20]. Heart rate variability is easy to perform, sensitive, reproducible, noninvasive and independent of patient's cooperation. HRV analysis can characterize and quantify variations in sympathetic and vagal activity. Hence , we have done HRV analysis to assess the autonomic function. Then we correlated P100 latency with the HRV indices. Expecting a negative correlation between P100 and SDNN, we observed a negative correlation, in diabetics but it was not statistically significant. LF and HF power also had a negative correlation with P100. There was a positive correlation between P100 and LF/HF. But in our study, the correlations in diabetics were not statistically significant.

CONCLUSION

HRV analysis in type 1 diabetes mellitus patients show

- Significant reduction in SDNN,RMSSD,NN50 and pNN50
- Significant reduction of HF and HF nu
- Significant increase in LF, LFnu and LF/HF ratio

HRV analysis can detect early subclinical alterations of the autonomic nervous system in asymptomatic patients with type 1 diabetes mellitus, which is mainly a parasympathetic impairment.

Early detection of cardiac autonomic dysfunction will help to motivate patients to improve their diabetes control and thereby delay the development of complications.

Our VEP data shows that there is a significant decrease in central nerve conduction in Type 1 Diabetic patients. They showed a significant prolongation of the latency of P 100 and N 75 and a reduction in amplitude of the VEP wave form. Therefore electrophysiological studies should be performed in type 1 diabetics in order to detect nervous system involvement.

Hence, all type 1 diabetic patients should be screened by HRV analysis and neuro-electrophysiological tests (VEP) so that the quality of life in these patients can be improved.

SUMMARY

The aim of the study was to evaluate the cardiovascular autonomic function and central nervous system alterations in Type 1 diabetes mellitus patients. The study group consisted of 30 Type 1 diabetes mellitus patients attending Diabetology OPD, SMC. The control group consisted of 30 healthy subjects matched for age, sex and BMI.

2 tests were performed- HRV and VEP

1. HRV analysis was done to evaluate Autonomic function. The following HRV indices were calculated- SDNN, RMSSD, NN50, pNN50 in the Time domain and LF, HF, LF/HF, LFnu and HFnu in the frequency domain.

Overall HRV as measured by SDNN was significantly reduced. There was a significant reduction in HF. LF power was significantly higher in diabetics compared to controls. All these observations taken together signify vagal neuropathy.

2. VEP was also recorded in the same subjects. There was a significant prolongation of the latencies of P100, N75 and there was a significant reduction in amplitude of the VEP waveform in the diabetic group compared to controls. This shows that there is delayed conduction in the optic pathways in diabetics.

This study has effectively shown that autonomic dysfunction can be detected very early, in the asymptomatic period, using heart rate variability analysis. It has also proved that VEP is a valuable method to detect early involvement of the optic pathway. Hence these tests can be considered as useful screening tests for type 1 diabetic patients.

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APPENDIX A

PROFORMA

PATIENT DATA COLLECTION

Department of Physiology, SMC, Chennai

Name		Age(yrs)		Gender	
I.D.No.		Date		Group	
Weight(Kg)		Height(cm)			

ADDRESS OF THE PATIENT :

MOBILE NUMBER

BLOOD PRESSURE

BLOOD SUGAR:Fasting Post-prandial

ONSET OF DISEASE

DURATION OF DISEASE

TREATMENT :

VISUAL ACUITY:

APPENDIX B

CLINICAL SYMPTOMS AND/ HISTORY PROFORMA

Autonomic Function Laboratory

Department of Physiology SMC, Chennai

I.D. number

No.	Autonomic Symptoms		Yes	No
1.	Nasal Symptoms	a.Dry nose b.Running nose		
2.	Sweating Symptoms	a.Increased b.Decreased		
3.	Postural fall/Dizziness on standing			
4.	GIT Symptoms	a.Diarrhoea b.Constipation c.Discomfort/pain		
5.	Headache/Migraine(Heaviness of head/throbbing headache)			
6.	Micturition disturbances	a.Frequency b.Urgency c.Incontinence d.Nocturia e.Hesitancy		

7.	Occasional attacks of Bronchospasm (after exercise, laughter, or emotion)						
8.	Do you often feel too hot or too cold						
9.	Do your extremities remain				a.Warm b.Cold		
10.	Burning feet						
11.	Risk factors				a. Obesity b.Hypertension c.CHD d..Smoking, Alcoholism		
12.	Any stress related physical symptoms (Flushing, choking, lump in the throat, general weakness)						
13.	Any other general symptoms						
14.	Allergy to any drug						
15.	Physical status	Regular athelete	Occ.player/ Sedentary	Regular exercise			

APPENDIX C

MEASURES OF HRV ANALYSIS

ID number

MEASURES	**LYING**	**STANDING**	**ONE MINUTE DEEP BREATHING**
FREQUENCYDOMAIN MEASURES 1.LF 2.HF 3.LF/HF RATIO 4.LF nu 5.HF nu 6.Total Power			
TIME DOMAIN MEASURES 1.SDNN 2.RMSSD 3.NN50 4.pNN50			

APPENDIX D

VEP DATA

ID number

	LEFT EYE	RIGHT EYE
N 75		
P 100		
N 145		
P 100 – N 75		

APPENDIX - E

CONSENT FORM

I Mr./Ms. /Mrs._____understand that Dr. xxxxxxx, a postgraduate student in Stanley Medical College and

Hospital, Chennai is doing this study on subjects with type 1 diabetes mellitus and on control group. I have been made to understand that these tests will assess the functioning of my heart and nervous system. These tests are simple; involve taking ECGs, and recording evoked potentials after visual stimulus. They do not involve injections or taking any medicines and are risk free. I have been familiarized with the testing procedures. I am participating in this study willingly. I have not been forced to do so. I have also been told clearly that I could withdraw from this study without any prejudice.

Date :

Signature :

MASTER CHART

															LEFT EYE			
S NO	ID NO	GROUP		AGE	GENDER	HEIGHT	WEIGHT	BMI	SBP	DBP	B SUGAR(F)	PPBS	ONSET OF DISEASE	DURATION	N75	P100	N145	P100-N75(mV)
1	J001	DM1		16	M	172	68	22.99	112	84	127	140	8	8	68.29	99.24	133.81	6.47
2	J002	DM1		37	M	167	67	24.02	122	80	125	138	21	16	71.63	103.29	146.25	4.07
3	J003	DM1		34	F	155	52	21.64	122	84	140	220	18	16	79.5	108.75	154.38	0.76
4	J004	DM1		32	F	142	46	22.81	130	80	132	200	15	17	76.38	99.25	138.27	5.82
5	J005	DM1		10	F	135	30	16.46	110	70	141	192	7	3	69.4	100.6	166.3	12.16
6	J006	DM1		27	M	168	45	15.94	116	82	122	134	22	5	69.38	92.5	157.5	3.89
7	J007	DM1		32	M	150	59	26.22	130	86	137	198	29	3	60	100.63	158.75	4.39
8	J008	DM1		25	F	152	56	24.24	124	86	180	240	22	3	70.63	100.63	142.5	11.31
9	J009	DM1		24	F	148	50	22.83	126	82	112	137	16	8	67.5	99.38	149.38	7.45
10	J010	DM1		30	F	153	49	20.93	124	82	126	180	25	5	63.75	88.13	141.88	8.67
11	J011	DM1		21	F	142	45	22.32	120	80	118	158	17	4	66.32	87.71	159.8	6.46
12	J012	DM1		20	F	152	55	23.81	122	76	96	132	15	5	69.38	99.38	168.13	7.91
13	J013	DM1		24	M	158	52	20.83	126	82	104	126	22	2	67.32	88.63	130.42	6.83
14	J014	DM1		23	M	162	55	20.96	110	70	110	128	19	4	70	100	142.5	3.5
15	J015	DM1		27	M	158	57	22.83	124	82	117	134	25	2	71.63	101.28	152.5	3.6
16	J016	DM1		22	M	162	55	20.96	118	76	145	182	7	15	78.34	97.12	131.41	5.92
17	J017	DM1		20	M	157	55	22.31	124	82	107	125	6	14	69.23	99.2	141.54	6.02
18	J018	DM1		15	F	143	45	22.01	112	70	130	162	11	4	76.25	101.25	153.75	9.73
19	J019	DM1		24	M	163	55	20.70	126	82	110	129	16	8	68.29	99.24	133.81	6.47
20	J020	DM1		22	F	151	46	20.17	120	80	124	142	10	12	71.21	102.14	146.27	5.48
21	J021	DM1		26	M	166	53	19.23	122	82	116	137	18	8	72.44	89.73	143.27	6.57
22	J022	DM1		20	F	155	58	24.14	120	80	102	131	14	6	70.63	99.3	159.38	5.1
23	J023	DM1		24	M	162	69	26.29	120	76	93	128	22	2	60	95	140.63	4.92
24	J024	DM1		18	M	161	54	20.83	116	80	114	144	10	8	65.63	88.13	145.63	4.06

25	J025	DM1		24	M	160	58	22.66	118	76	115	147	18	6	68.75	91.25	140	7.07
26	J026	DM1		12	F	139	48	24.84	112	70	132	160	10	2	66.88	96.25	150	5.4
27	J027	DM1		23	M	158	60	24.03	126	76	152	192	15	8	68.75	99.38	153.75	2.23
28	J028	DM1		19	M	157	55	22.31	124	82	136	171	13	6	66.25	86.25	140.42	5.57
29	J029	DM1		21	M	163	57	21.45	126	84	143	180	17	4	61.25	97.5	143.75	4.72
30	J030	DM1		20	M	168	61	21.61	116	80	116	145	14	6	64.38	95.25	145.7	4.11

			RIGHT EYE				SUPINE REST											
S NO	**ID NO**	**GROUP**	**N75**	**P100**	**N145**	**P100-N75(Mv)**	**MEAN RR**	**MEAN HR**	**SDNN**	**RMSSD**	**NN50**	**pNN50**	**LF in ms2**	**HF in ms2**	**LF/HF**	**LF(n.u)**	**HF(n.u)**	**total power**
1	J001	DM1	69.01	99.76	134.18	6.58	0.703	85	25.424	14.642	1	0.3	6.4	1.9	3.368	77.1	22.9	8.3
2	J002	DM1	83.13	106.88	146.25	4.07	0.683	88	22.26	11.582	3	0.7	19.3	3	6.433	86.5	13.5	22.3
3	J003	DM1	83.75	113.13	149.23	1.96	0.645	93	11.648	5.416	0	0	19.2	8.3	2.313	69.8	30.2	27.5
4	J004	DM1	74.24	99.82	139.32	5.92	0.714	84	18.43	11.688	0	0	12.7	5.1	2.49	71.8	28.8	17.8
5	J005	DM1	70	100.6	168.1	10.63	0.61	98	34.875	26.93	30	6.1	44.4	30.2	1.47	59.5	40.5	74.6
6	J006	DM1	69.38	93.13	158.13	4.68	0.925	65	33.839	33.303	45	13.9	2.2	2.6	0.846	45.8	54.2	4.8
7	J007	DM1	65	101.38	157.75	6.62	0.649	92	11.804	5.323	0	0	25	8.4	2.976	74.9	25.1	33.4
8	J008	DM1	71.25	100.63	141.88	10.73	0.679	88	21.919	10.374	0	0	22.4	6.5	3.446	77.5	22.5	28.9
9	J009	DM1	67.5	99.38	150	7.03	0.925	65	32.944	31.657	39	12.1	6.4	1.9	3.368	77.1	22.9	8.3
10	J010	DM1	64.38	88.75	141.88	9.05	0.679	88	24.473	12.122	3	0.7	21.6	7.1	3.042	75.3	24.7	28.7
11	J011	DM1	67.2	87.2	158.9	6.45	0.689	87	59.006	26.795	27	6.4	15.4	8.1	1.901	65.5	34.5	23.5
12	J012	DM1	68.75	96.88	163.13	8.67	0.708	85	24.721	20.31	4	1	13	5.9	2.203	69.1	31.4	18.9
13	J013	DM1	68.12	87.25	130.42	6.79	0.648	93	31.451	16.944	8	1.7	53.5	10.9	4.908	83.2	17	64.4
14	J014	DM1	70	100	143.75	4.07	0.667	90	66.154	24.649	24	5.5	38.7	4.4	8.795	89.8	10.2	43.1
15	J015	DM1	70.63	101.88	152.5	4.07	0.642	93	29.742	34.184	7	1.5	38.4	10.1	3.802	79.3	20.9	48.5
16	J016	DM1	77.22	96.77	130.2	5.87	0.586	102	13.129	6.222	0	0	15.3	3.7	4.135	80.5	19.5	19

17	J017	DM1	70.48	99.7	141.78	5.98	0.635	94	62.957	37.482	52	11.5	39.6	13.1	3.023	75.1	24.9	52.7
18	J018	DM1	75.63	101.25	153.13	7.25	0.709	85	24.836	20.739	7	1.7	11.6	5.1	2.274	69.5	30.5	16.7
19	J019	DM1	69.01	99.76	134.18	6.58	0.709	85	21.925	36.55	1	0.2	18.3	5.1	3.588	70.2	21.8	23.4
20	J020	DM1	69.63	99.75	144.6	5.92	0.797	75	20.255	13.465	0	0	7.8	2.3	3.391	77.2	22.8	10.1
21	J021	DM1	72.63	89.21	142.28	6.62	0.62	97	15.6	13.231	0	0	45	15.3	2.941	74.6	25.4	60.3
22	J022	DM1	69.38	98.75	150.63	4.98	0.703	85	29.922	38.803	7	1.6	15.5	5.6	2.768	73.5	26.5	21.1
23	J023	DM1	60	90	136.88	3.04	0.693	87	37.489	39.039	11	2.5	43.2	4.7	9.191	90.4	9.8	47.9
24	J024	DM1	68.13	91.25	142.88	3.6	0.62	97	15.919	31.35	1	0.2	42.8	19.2	2.229	69	31	62
25	J025	DM1	68.13	93.13	141.25	6.96	0.702	85	30.968	38.087	7	1.6	13	6.4	2.031	67	33	19.4
26	J026	DM1	65.75	96.25	150	5.47	0.638	94	30.298	34.944	2	0.4	30.2	13	2.323	69.9	30.1	43.2
27	J027	DM1	68.74	95.63	154.38	3.57	0.684	88	25.088	12.747	4	0.9	18.3	3.3	5.545	84.7	15.3	21.6
28	J028	DM1	65.63	86.25	142.5	4.55	0.658	91	28.494	37.1	5	1.1	31.4	11.6	2.707	73.2	27	43
29	J029	DM1	66.25	98.13	143.13	4.71	0.712	84	22.32	36.563	1	0.2	16.2	5.3	3.057	75.3	24.7	21.5
30	J030	DM1	64.38	95	145	4.12	0.79	76	28.459	52.226	3	0.8	9.5	2.9	3.276	76.6	23.4	12.4

			STANDING											**DEEP BREATHING**							
S NO	**ID NO**	**GROUP**	**MEANRR**	**MEANHR**	**SDNN**	**RMSSD**	**NN50**	**Pnn50**	**LF in ms2**	**HF in ms2**	**LF/HF**	**LF(n.u)**	**HF(n.u)**	**total power**	**MEANRR**	**MEANHR**	**SDNN**	**RMSSD**	**NN50**	**pNN50**	**HF in ms2**	**HF(n.u)**
1	J001	DM1	0.607	99	27.982	10.441	0	0	56.7	3.7	15.324	93.9	6.1	60.4	0.792	76	70.639	35.692	10	14.5	0.1	5.6
2	J002	DM1	0.649	92	25.924	17.592	6	1.6	8.5	2.1	4.048	80.2	19.8	10.6	0.661	91	85.652	127.462	25	53.2	0.3	16.7
3	J003	DM1	0.598	100	7.777	3.943	0	0	35.1	4	8.775	89.8	10.2	39.1	0.676	89	32.973	17.933	1	1.1	0.5	19.2
4	J004	DM1	0.608	99	31.976	11.156	1	0.2	59	3.9	15.128	93.9	6.2	62.9	0.792	76	70.639	35.692	10	14.5	0.1	5.6
5	J005	DM1	0.571	105	43.744	37.9	56	11	33.2	12.1	2.744	73.3	26.7	45.3	0.62	97	59.657	72.665	17	40.5	1.9	27.9
6	J006	DM1	0.767	78	32.559	18.066	5	1.3	14.9	2.7	5.519	84.7	15.3	17.6	0.904	66	65.403	39.618	14	21.5	0.2	11.1
7	J007	DM1	0.604	99	23.106	7.245	2	0.4	35.7	4.9	7.286	87.9	12.1	40.6	0.676	89	32.973	17.933	1	1.1	0.5	19.2
8	J008	DM1	0.623	96	22.912	10.103	0	0	45.8	12.5	3.664	78.6	21.4	58.3	0.745	81	28.926	18.265	0	0	0.2	16.7
9	J009	DM1	0.759	79	32.776	17.084	5	1.3	15.9	3.1	5.129	83.7	16.3	19	0.904	66	65.403	39.618	14	21.5	0.2	11.1

10	J010	DM1	0.618	97	19.691	10.274	0	0	44.6	11.1	4.018	80.1	19.9	55.7	0.745	81	28.926	18.265	0	0	0.2	16.7
11	J011	DM1	0.544	110	17.999	8.885	0	0	72.9	10.4	7.01	87.5	12.5	83.3	0.687	87	58.139	62.518	19	39.6	0.1	9.1
12	J012	DM1	0.599	100	20.665	10.291	0	0	45.5	5.4	8.426	89.2	10.6	50.9	0.735	82	63.645	30.883	3	6	0.3	15
13	J013	DM1	0.567	106	51.691	27.317	36	6.8	70.1	12.9	5.434	84.5	15.5	83	0.622	96	44.884	63.012	2	2.1	4	16.7
14	J014	DM1	0.546	110	18.088	8.73	0	0	74.8	8.5	8.8	89.8	10.2	83.3	0.687	87	58.139	62.518	19	39.6	0.1	9.1
15	J015	DM1	0.571	105	56.241	40.27	40	7.6	63.5	14	4.536	81.9	18.1	77.5	0.622	96	44.884	63.012	2	2.1	4	16.7
16	J016	DM1	0.552	109	15.844	7.524	0	0	74.9	5.8	12.914	92.8	7.2	80.7	0.676	89	32.973	17.933	1	1.1	0.5	19.2
17	J017	DM1	0.636	94	41.725	31.153	40	9.4	32.7	9.6	3.406	77.3	22.7	42.3	0.649	92	31.269	35.62	12	16.7	1.5	22.1
18	J018	DM1	0.605	99	22.91	11.291	0	0	39.4	7.2	5.472	84.5	15.5	46.6	0.735	82	63.645	30.883	3	6	0.3	15
19	J019	DM1	0.684	88	24.517	36.26	1	0.2	24.9	12.8	1.945	66.2	34	37.7	0.732	82	37.793	86.841	4	4.9	1	30.3
20	J020	DM1	0.589	102	13.052	26.522	1	0.2	37.1	5.7	6.509	86.7	13.3	42.8	0.847	71	73.799	95.919	19	26.8	0.8	42.1
21	J021	DM1	0.57	105	20.556	14.837	2	0.4	46.9	14.8	3.169	76	24	61.7	0.685	88	22.493	29.232	4	7.8	0.4	16
22	J022	DM1	0.553	108	26.178	26.058	2	0.4	60.2	9	6.689	87	13	69.2	0.786	76	45.707	97.986	3	3.9	0.3	20
23	J023	DM1	0.618	97	45.766	38.025	20	4.1	67.5	7.6	8.882	89.9	10.1	75.1	0.67	90	38.455	68.273	5	5.3	1.1	8.8
24	J024	DM1	0.574	105	22.038	14.54	2	0.4	69	15.3	4.51	81.8	18.1	84.3	0.685	88	22.493	29.232	4	7.8	0.4	16
25	J025	DM1	0.557	108	31.97	26.821	4	0.7	63.7	8.3	7.675	88.5	11.5	72	0.786	76	45.707	97.986	3	3.9	0.3	20
26	J026	DM1	0.503	119	18.701	21.791	1	0.2	27.4	4.1	6.683	87.3	13.1	31.5	0.727	83	66.62	92.026	17	20.7	0.9	23.1
27	J027	DM1	0.654	92	29.19	11.101	1	0.2	51.3	5.3	9.679	90.8	9.4	56.6	0.685	88	22.493	29.232	4	7.8	0.4	16
28	J028	DM1	0.565	106	39.188	30.127	9	1.6	29.1	26.5	1.098	52.3	47.7	55.6	0.693	87	84.139	99.8	23	25.8	2.2	29.3
29	J029	DM1	0.683	88	23.351	37.436	1	0.2	28.7	12.7	2.26	69.3	30.7	41.4	0.732	82	37.793	86.841	4	4.9	1	30.3
30	J030	DM1	0.591	102	31.854	31.152	3	0.6	48.4	5.7	8.491	89.5	10.5	54.1	0.847	71	73.799	95.919	19	26.8	0.8	42.1

															LEFT EYE			
S NO	ID NO	GROUP		AGE	GENDER	HEIGHT	WEIGHT	BMI	SBP	DBP	B SUGAR(F)	PPBS			N75	P100	N145	P100-N75(mV)
31	S001	CONTROL		21	F	155	53	22.06	110	70	83	104			65.63	90	153.75	4.2
32	S002	CONTROL		30	F	157	56	22.72	124	80	91	105			63.75	90.1	161.75	3.72
33	S003	CONTROL		25	F	159	52	20.57	112	80	84	104			60	90.63	133.75	7.52
34	S004	CONTROL		10	F	129	28	16.83	100	70	73	102			62.4	87.3	132.63	7.53
35	S005	CONTROL		32	F	154	60	25.30	116	84	82	104			70.63	83.75	142.5	7.81
36	S006	CONTROL		34	F	152	55	23.81	110	70	86	103			68.4	89.2	157.4	6.89
37	S007	CONTROL		17	F	155	53	22.06	124	82	81	102			67.5	90	166.3	5.87
38	S008	CONTROL		15	F	142	45	22.32	120	80	79	103			65.63	92.5	145	3.74
39	S009	CONTROL		20	F	154	56	23.61	112	76	82	108			60	93.75	173.75	9.13
40	S010	CONTROL		19	M	176	68	21.95	130	84	80	106			60.63	87.5	140.63	5.41
41	S011	CONTROL		18	M	173	55	18.38	126	86	76	107			64.38	94.38	153.13	10. 84
42	S012	CONTROL		24	F	149	50	22.52	114	76	86	110			65.12	91.5	151.4	8.24
43	S013	CONTROL		22	F	148	46	21.00	110	70	81	108			64.2	90.2	148.4	7.25
44	S014	CONTROL		13	F	142	47	23.31	116	82	85	112			61.88	83.13	139.75	7.25
45	S015	CONTROL		20	M	160	44	17.19	112	82	78	106			68.75	84.2	140.63	8.14
46	S016	CONTROL		22	M	167	68	24.38	126	80	81	105			67.15	90.2	161.5	6.87
47	S017	CONTROL		20	M	150	51	22.67	116	82	88	109			65.1	87.4	141.25	8.17
48	S018	CONTROL		16	M	160	57	22.27	118	76	81	105			70.2	89.4	143.5	7.25
49	S019	CONTROL		23	M	158	60	24.03	120	80	92	114			68.4	92.5	140	6.18
50	S020	CONTROL		35	M	150	53	23.56	126	82	84	111			69.2	91.4	142.5	7.15
51	S021	CONTROL		27	M	153	50	21.36	118	74	88	112			70.15	90.5	143.5	9.5
52	S022	CONTROL		32	M	169	61	21.36	122	82	80	114			68.45	91.2	144.2	7.3
53	S023	CONTROL		25	M	168	50	17.72	126	82	83	113			67.5	89.5	145.2	6.65

54	S024	CONTROL		26	M	162	56	21.34	116	80	77	108			71.5	88.5	146.4	7.15
55	S025	CONTROL		23	M	158	57	22.83	124	82	80	107			68.75	89.2	144.8	8.25
56	S026	CONTROL		26	M	163	56	21.08	126	82	79	105			68.4	89.35	143.5	7.4
57	S027	CONTROL		24	M	162	67	25.53	112	70	81	112			69.4	89.4	142.9	6.1
58	S028	CONTROL		24	M	161	55	21.22	124	80	85	114			68.5	89.5	143.6	7.2
59	S029	CONTROL		21	M	160	58	22.66	120	76	78	112			70.3	89.6	143.9	7.15
60	S030	CONTROL		22	M	158	60	24.03	110	70	86	113			69.5	90.1	148	6.45

			RIGHT EYE				SUPINE REST											
S NO	**ID NO**	**GROUP**	**N75**	**P100**	**N145**	**P100-N75(Mv)**	**MEAN RR**	**MEAN HR**	**SDNN**	**RMSSD**	**NN50**	**pNN50**	**LF in ms2**	**HF in ms2**	**LF/HF**	**LF(n.u)**	**HF(n.u)**	**total power**
31	S001	CONTROL	65.63	90	154.38	3.75	0.846	71	104.74	88.956	171	48.3	9.8	24.8	0.395	28	71.7	34.6
32	S002	CONTROL	68.13	90.63	160	3.81	0.909	66	60.457	88.728	155	58.9	3.8	3.6	1.056	51	48.6	7.4
33	S003	CONTROL	63.75	93.73	136.25	6.89	0.99	61	110.604	150.148	220	75.9	5.1	5.9	0.864	46	53.6	11
34	S004	CONTROL	64.39	90.5	131.75	7.42	1.014	59	43.518	38.539	47	16	4.1	1.5	2.733	73	26.8	5.6
35	S005	CONTROL	70.63	83.75	142.5	8.34	0.71	85	28.908	28.268	28	6.6	15.9	14.3	1.112	53	47.4	30.2
36	S006	CONTROL	69.2	89.12	156.5	7.02	1.02	59	54.873	79.394	201	68.6	1.8	6.2	0.29	23	77.5	8
37	S007	CONTROL	67.5	89.4	166.3	5.85	0.925	65	33.839	33.303	45	13.9	2.2	2.6	0.846	46	54.2	4.8
38	S008	CONTROL	65.63	91.25	136.25	3.39	0.686	87	94.081	115.227	183	42.7	15.6	62.9	0.248	20	80.1	78.5
39	S009	CONTROL	66.88	98.75	173.75	10.61	0.935	64	56.315	97.558	194	60.6	4.1	4.8	0.854	46	53.9	8.9
40	S010	CONTROL	62	89.3	142.5	4.34	0.642	93	21.979	35.017	2	0.4	54.4	19.6	2.776	74	26.5	74
41	S011	CONTROL	64.38	95	153.13	12.3	0.884	68	56.808	85.666	141	41.6	11.9	6.7	1.776	64	36	18.6
42	S012	CONTROL	64.9	90.15	150.4	8.5	0.844	71	104.671	101.213	172	48.5	10.2	23.6	0.432	30	69.8	33.8
43	S013	CONTROL	64.5	89.9	147.2	7.45	0.83	72	36.134	48.409	10	2.8	6	2.2	2.727	73	26.8	8.2
44	S014	CONTROL	62.35	85.23	132.8	7.38	0.773	78	78.059	85.023	208	53.6	17.1	33.6	0.509	34	66.4	50.7
45	S015	CONTROL	71.88	85.15	143.75	6.24	0.749	80	47.859	52.79	65	16.3	15.9	12	1.325	57	43	27.9

46	S016	CONTROL	67.5	89.4	158.5	6.65	0.773	78	71.428	64.494	169	43.3	13.3	16.1	0.826	45	54.8	29.4
47	S017	CONTROL	72.45	88.25	137.75	6.58	0.676	89	26.57	37.768	11	2.5	23.5	20.8	1.13	53	47	44.3
48	S018	CONTROL	71.4	89.6	143.9	7.15	0.658	91	28.494	37.1	5	1.1	31.4	11.6	2.707	73	27	43
49	S019	CONTROL	79.2	93.14	138.75	6.12	0.675	89	27.509	38.638	15	3.4	19.9	28	0.711	42	58.3	47.9
50	S020	CONTROL	69.5	90.6	145.75	7.05	0.684	88	48.009	63.689	110	25	19.9	40	0.498	33	66.8	59.9
51	S021	CONTROL	71.25	89.95	142.8	8.75	0.606	99	53.292	54.406	41	8.3	37.2	22.7	1.639	62	37.9	59.9
52	S022	CONTROL	69.2	90.5	143.45	7.15	0.896	67	54.144	63.714	136	42.5	8.7	6.1	1.426	59	41.2	14.8
53	S023	CONTROL	68.1	88.6	145.5	6.8	1.012	59	55.77	90.857	199	67.2	2.3	2.9	0.793	43	54.7	5.2
54	S024	CONTROL	72.4	88.4	149.2	7.45	0.926	65	60.311	81.85	153	47.4	4	3.3	1.212	56	45.8	7.3
55	S025	CONTROL	68.6	89.35	143.5	8.5	0.673	89	48.254	56.637	126	28.3	35.2	35.2	1	50	50	70.4
56	S026	CONTROL	68.5	89.2	142.6	7.6	0.722	83	59.785	45.608	46	11	11.2	13.5	0.83	45	54.7	24.7
57	S027	CONTROL	69.2	89.5	141.9	6.5	0.937	64	61.647	91.871	203	64	4.7	2.3	2.043	67	32.9	7
58	S028	CONTROL	68.7	88.9	144.5	6.9	0.681	88	50.505	68.676	109	24.7	24.1	24	1.004	50	49.9	48.1
59	S029	CONTROL	71.4	89.5	144.8	7.25	0.776	77	72.38	70.911	193	50	13.7	23.5	0.583	37	63	37.2
60	S030	CONTROL	70.2	89.5	149.2	6.8	0.681	88	47.629	62.728	147	33.3	28.4	42.3	0.671	40	59.8	70.7

			STANDING												DEEP BREATHING							
S NO	ID NO	GROUP	MEANRR	MEANHR	SDNN	RMSSD	NN50	Pnn50	LF in ms2	HF in ms2	LF/HF	LF(n.u)	HF(n.u)	total power	MEANRR	MEANHR	SDNN	RMSSD	NN50	pNN50	HF in ms2	HF(n.u)
31	S001	CONTROL	0.799	75	87.887	65.825	171	45.6	14.7	13.1	1.122	52.9	47.1	27.8	0.894	67	117.29	78.256	16	24.2	6.3	48.8
32	S002	CONTROL	0.761	79	63.703	51.51	72	18.3	9.2	6.2	1.484	59.7	40.3	15.4	0.89	68	49.17	135.43	34	50.7	2	38.5
33	S003	CONTROL	0.825	73	74.94	97.05	212	58.6	12.2	7.7	1.58	61.3	38.7	19.9	0.93	64	93.53	98.24	50	60.2	2.2	21.6
34	S004	CONTROL	0.9	67	77.864	34.957	23	6.9	6.3	1.6	3.938	80.8	20.5	7.9	1.01	59	70.722	51.576	18	31.6	2.4	18.5
35	S005	CONTROL	0.652	92	27.396	22.069	10	2.2	24.3	28.7	0.847	45.9	54.3	53	0.714	84	119.184	123.262	40	53.3	4.4	36.4
36	S006	CONTROL	0.785	76	35.242	25.293	20	5.3	9.7	3.4	2.853	74	26	13.1	1.073	56	111.109	80.465	25	48.1	0.2	40
37	S007	CONTROL	0.767	78	32.559	18.066	5	1.3	14.9	2.7	5.519	84.7	15.3	17.6	0.904	66	65.403	39.618	14	21.5	0.2	11.1
38	S008	CONTROL	0.552	109	39.476	34.055	23	4.5	40.5	34.9	1.16	53.7	46.3	75.4	0.714	84	119.184	123.262	40	53.3	4.4	36.4
39	S009	CONTROL	0.825	73	74.941	97.054	212	58.6	12.2	7.7	1.584	61.3	38.7	19.9	0.934	64	93.527	98.238	50	60.2	2.2	21.6
40	S010	CONTROL	0.555	108	39.476	34.055	23	4.5	40.5	34.9	1.16	53.7	46.3	75.4	0.714	84	119.184	123.262	40	53.3	4.4	36.4
41	S011	CONTROL	0.733	82	73.643	40.473	88	22.2	28.1	3.6	7.806	88.6	11.4	31.7	0.941	64	81.96	92.883	38	64.4	0.1	10
42	S012	CONTROL	0.797	75	88.057	74.217	172	45.7	14.9	13	1.146	53.4	46.6	27.9	0.894	67	117.29	78.256	16	24.2	6.3	48.8
43	S013	CONTROL	0.753	80	37.039	41.749	3	0.8	11.6	1.3	8.923	89.9	10.1	12.9	0.858	70	67.76	113.821	9	12.9	0.6	25
44	S014	CONTROL	0.677	89	43.068	41.685	41	9	41.4	23.2	1.784	64.1	35.9	64.6	0.79	76	101.047	115.835	28	36.4	2.2	38.6
45	S015	CONTROL	0.615	98	26.292	13.039	2	0.3	53.3	16.2	3.29	76.7	23.3	69.5	0.774	78	92.691	116.077	20	26	0.2	22.2
46	S016	CONTROL	0.674	89	43.949	28.255	34	7.8	41.5	17.2	2.413	70.7	29.3	58.7	0.858	70	67.76	113.821	9	12.9	0.6	25
47	S017	CONTROL	0.567	106	34.064	31.219	6	1.1	30.2	22	1.373	57.9	42.1	52.2	0.691	87	83.865	81.644	22	25	0.7	20.6
48	S018	CONTROL	0.565	106	39.188	30.127	9	1.6	29.1	26.5	1.098	52.3	47.7	55.6	0.693	87	84.139	99.8	23	25.8	2.2	29.3
49	S019	CONTROL	0.551	109	29.958	27.635	5	0.9	31.1	31.6	0.984	49.6	50.4	62.7	0.701	86	84.507	82.847	25	28.4	0.9	27.3
50	S020	CONTROL	0.657	91	45.658	37.439	24	5.3	52.5	9.2	5.707	85.1	14.9	61.7	0.733	82	65.48	96.901	32	39	1.4	16.5
51	S021	CONTROL	0.538	112	49.651	22.129	21	3.9	74.9	6.6	11.348	91.9	8.1	81.5	0.641	94	72.836	50.361	15	17.2	1.3	8.8
52	S022	CONTROL	0.73	82	73.64	40.47	88	22.2	28.1	3.6	7.81	88.6	11.4	31.7	0.94	64	81.96	92.88	38	64.4	0.1	10
53	S023	CONTROL	0.778	77	39.393	47.791	21	5.4	8.2	3.6	2.278	69.5	30.5	11.8	1.073	56	111.11	80.47	25	48.1	0.2	40

54	S024	CONTROL	0.762	79	51.324	50.427	60	15.2	11.4	6.6	1.727	63.3	36.7	18	0.888	68	49.174	135.425	34	50.7	2	38.5
55	S025	CONTROL	0.591	102	29.697	34.085	11	2.1	28	29.2	0.959	49	51.1	57.2	0.76	79	95.208	116.666	27	34.2	2	41.7
56	S026	CONTROL	0.62	98	26.29	13.04	2	0.3	53.3	16.2	3.29	76.7	23.3	69.5	0.77	78	92.69	116.08	20	26	0.2	22.2
57	S027	CONTROL	0.854	70	87.13	84.919	197	59.2	8.2	3.3	2.485	71.9	28.9	11.5	0.934	64	93.52	98.23	50	60.2	2.2	21.6
58	S028	CONTROL	0.653	92	42.367	37.443	20	4.3	53.2	9.3	5.72	85.1	14.9	62.5	0.7	82	65.5	96.9	32	39	1.4	16.5
59	S029	CONTROL	0.677	89	42.102	28.679	37	8.3	41.2	22.2	1.856	65	35	63.4	0.79	76	101.05	115.84	28	36.4	2.2	38.6
60	S030	CONTROL	0.603	100	38.355	37.316	28	5.6	47.7	23.4	2.038	67.1	32.9	71.1	0.76	79	95.21	116.67	27	34.2	2	41.7